ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 391

EVALUATION OF THE EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON SEROTONIN AND CATECHOLAMINE NEURONS IN THE RAT CNS

BY

H. G. BAUMGARTEN A. BJÖRKLUND L. LACHENMAYER and A. NOBIN

LUND 1973

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 391

FROM THE DEPARTMENTS OF ANATOMY AND NEUROANATOMY
UNIVERSITY OF HAMBURG, G.F.R. AND DEPARTMENT OF HISTOLOGY
UNIVERSITY OF LUND SWEDEN

EVALUATION OF THE EFFECTS OF 5,7-DIHYDROXYTRYPTAMINE ON SEROTONIN AND CATECHOLAMINE NEURONS IN THE RAT CNS

BY

H. G. BAUMGARTEN A. BJÖRKLUND L. LACHENMAYER
and A. NOBIN

Abbreviations used 5,7-DHT 5,7-dihydroxytryptamine 5,6-DHT 5,6-dihydroxytryptamine 5-HT 5-hydroxytryptamine (serotonin); CA, catecholamines NA, noradrenaline DA dopamine IA, indolamines.

Printed in Sweden
Lund 1973 AB Grahms Boktryckeri

SUMMARY

Doses of 10—200 μg (free base) of 5 7 dihydroxytryptamine (5 7 DHT) injected into the lateral ventricle induced a dose-dependent significant long lasting reduction of serotonin and noradrenaline in rat brain and spinal cord The time-course of depletion and recovery of brain serotonin was analysed in individual brain regions between 3 hrs and 45 days after a single high dose of 5 7 DHT (200 μg) In ventricle near regions (septum and hypothalamus) as well as in the cell body rich parts of the rat CNS (mesencephalon and pons + medulla oblongata) a profound reduction in 5-HT (up to 80 %) was registe red by 3—24 hrs. After a transient recovery phase with somewhat higher 5-HT concentrations at 4 days (35—55 % of control) the 5-HT levels declined to near minimum values (15—45 % of control) at 10 days. Thereafter a slight non-significant recovery of 5-HT occurred between 10 and 45 days. Spinal cord, the forebrain-rest sample and striatum differed in their response to 5 7 DHT from the other brain regions, and revealed a more gradual and retarded loss of 5-HT which resulted in minimum values (10—25 % of control) at 10 days. The levels of serotonin then remained depressed up to 45 days.

10 days after 10—200 μg 5 7 DHT whole brain dopamine concentrations did not differ from control In contrast, whole brain and spinal cord nora drenaline levels showed a dose dependent reduction 10 days after 10—200 μg 5 7 DHT Following a single high dose (200 μg) of 5 7 DHT there was a rapid reduction in spinal cord and whole brain noradrenaline to approximately 25 % of control at 3 hrs. During the subsequent four days there was a partial recovery in the brain noradrenaline concentration to 35—55 % of control at 4—45 days. In the spinal cord there was a transient recovery at 1—4 days, and maximum reduction (about 15 % of control) was reached by 10 and 45 days.

The long lasting depletion of brain and spinal cord 5-HT and noradrenaline concentrations was — at 9—11 days after 200 μg of 5 7 DHT — accompanied by a strong reduction in the ability of slices from brain and spinal cord to accumulate ^{3}H 5-HT (85—90 % reduction) and ^{3}H noradrenaline (40—60 % reduction) The fluorescence histochemical observations revealed an almost complete long term disappearance of indolamine-containing terminals, and to a lesser extent also noradrenaline-containing terminals, in several regions of the brain and spinal cord. In addition, there were fluorescence microscopical signs of extensive axonal damage in the major indolamine and noradrenaline axon bundles.

The results provide evidence that 5 7 DHT has a neurotoxic mode of action on serotonin neurons, and to a lesser extent also on noradrenaline neurons in the CNS.

It seems now well documented that 5 6-dihydroxytryptamine (5 6-DHT) [illegible] injected into the cerebrospinal fluid, causes degeneration of — primarily ind[illegible]lamine-containing axons and axon terminals. This neurotoxic mode of [illegible] of 5 6-DHT has been observed as a long-lasting depletion of brain [illegible] spinal cord serotonin levels (Baumgarten, Björklund, Lachenmayer Nobin [illegible] Stenevi 1971 Baumgarten, Evetts, Holman, Iversen, Vogt and Wilson [illegible] accompanied by ultrastructural signs of degeneration of non-myelinated [illegible] Baumgarten, Björklund, Holstein and Nobin 1972) by a reduction [illegible] [illegible]-HT uptake sites (Björklund Nobin and Stenevi 1973 a, b Daly Fuxe [illegible] J[illegible]sson 1973) and by a disappearance of fluorescence histochemically [illegible] the indolamine-containing axons and axon terminals (Baumgarten, [illegible]mayer and Schlossberger 1972, Björklund, Nobin and Stenevi 1973 a, b, [illegible] Baumgarten, Björklund, Lachenmayer and Stenevi 1973) With doses [illegible] 5 µg the degenerative effect of intraventricularly administered 5 6-DHT — with respect to the monoamine neuron systems at least — remarkably selective for the indolamine neurons 5 6-DHT has been found to cause transient reduction in brain NA and DA levels (Baumgarten *et al* 1971) but this depletory action of 5 6-DHT on CA neurons is only to a minor extent accompanied by axonal degeneration (Björklund *et al.* 1973 a, Nobin *et al* 1973)

When administered intraventricularly however 5 6-DHT has definite limitations as a tool for total and selective destruction of the indolamine-containing axons in the brain. Above all, a good selectivity of its neurotoxic effects is only obtained with doses up to 50—75 µg. With these doses an almost total destruction of the indolamine fibres is obtained only in the spinal cord, whereas in the brain the reduction in 5-HT and ^{3}H 5-HT uptake varies between 35 % and 65 % in different regions (Baumgarten *et al.* 1971 Björklund *et al.* 1973 a, b) Doses higher than 75 µg proved to be severely toxic to the animals, causing muscle paralysis, convulsions and tremor and most animals did not survive. Already after 75 µg, and more pronounced after 100 µg of 5,6-DHT the fluorescence and electron microscopic investigations have given evidence of a non-selective damage to myelinated fibre systems and a shrinkage of the striatum on the side of the injections, and there is a deposition of a brownish (probably melanin) pigment on the walls of the ventricles and the aqueduct (Baumgarten *et al* 1972 a, b, Nobin *et al.* 1973) Björklund, Nobin and Stenevi (1973 c) have demonstrated that this limitation in the use of 5,6-DHT — when administered intraventriculary — can be overcome by injecting small amounts of the drug directly into the brain parenchyma. Furthermore, this mode of administration allows more complete denervations of many brain regions, and thus after injection of 4 µg of 5,6-DHT directly into the ventromedial midbrain tegmentum (i.e. close to the major ascending

indolamine fibre bundles) forebrain 5-HT levels and ^{3}H 5-HT uptake were reduced by as much as 75 % and 60—70 % respectively (Bjorklund *et al.* 1973 c)

The discovery of the neurotoxic properties of 5 6-DHT prompted us to test other di and monohydroxylated tryptamines as tools for chemical degeneration of central indolamine neurons. The drawbacks of 5,6-DHT seem at least partly to be related to its high tendency to become oxidized at physiological pH. The more stable isomere, 5 7-dihydroxytryptamine (5 7 DHT) should therefore be of particular interest, as the greater chemical stability of this compound should give it better diffusion properties and, perhaps, less general toxicity Preliminary fluorescence microscopic (Baumgarten and Lachenmayer 1972) and biochemical studies (Baumgarten, Victor and Lovenberg 1973) have, in fact, provided evidence for a high neurotoxic potency of 5 7 DHT In the present investigation the possible neurotoxic effect of 5 7 DHT on monoamine-containing neurons have been evaluated in greater detail in the rat CNS

MATERIAL AND METHODS

Adult female albino rats (180—220 g) were given single injections of 10—200 µg free base of 5 7-dihydroxytryptamine (creatinine sulfate H_2O complex) into the lateral ventricle, under ether anaesthesia. The drug was dissolved in sterile saline containing 1 0 mg/ml ascorbic acid. The injection volume was 20 µl. In order to protect the animals from convulsions, they were treated with 40 mg/kg, *i.p.* of Nembutal® (Abbot) immediately after the 5 7 DHT injection. Nembutal was required only for animals receiving more than 50 µg 5 7 DHT The animals were killed by decapitation at times varying between 45 min and 45 days after the administration of 5 7 DHT

Monoamine determinations were carried out on either whole brains (olfactory bulbs and pineal gland removed) and spinal cord, or on pooled brain regions, dissected according to Baumgarten *et al.* (1971) 5-Hydroxytryptamine (5-HT) was determined fluorometrically according to Bertler (1961) and noradrenaline (NA) and dopamine (DA) according to Bertler Carlsson, Rosengren and Waldeck (1958) as modified by Häggendal (1963)

For the regional determinations of 5-HT regions from 4—5 rats were pooled for each sample. The number of samples assayed per time point were 8 samples from control animals, 4 samples from animals with a survival time of 3 and 24 hrs, and 4 10 and 30 days, and 2 samples from 45-day animals. In the dose—response experiments 5-HT was determined on single whole brains and spinal cords, 4—5 animals being used for each point. NA and DA were assayed on single whole brain or spinal cord and the number of samples per time point were 4 samples for each time point except for 45 days where three animals were available for the determinations. The results are expressed as % of control.

Uptake of ^{3}H 5-HT and ^{3}H-NA was measured *in vitro* on thin circular

slices (3 mm in diameter and 0.5—1.0 mm thick) prepared from cortex, hypothalamus and thoracic segments of the spinal cord according to Hamberger (1967) and Sachs and Jonsson (1972). The slices were incubated as described by Björklund, Nobin and Stenevi (1973 a), the procedure being as follows. After *preincubation for 10 min at +37°C* in a Krebs-Ringer bicarbonate buffer (pH 7.4, saturated with 95 % O_2 and 5 % CO_2, and containing 1.8 g/l glucose) ^{3}H 5-HT (^{3}H 5-HT creatinine sulphate, 6.8 Ci/mmole, gen. labelled, Radiochemical Centre, Amersham, England) or ^{3}H NA (L NA 7-^{3}H, 6.4 Ci/mmole, New England Nuclear, Frankfurt, G.F.R.) was added to a *final concentration of 0.5×10⁻⁷ M (^{3}H 5-HT) or 10⁻⁷ M (^{3}H NA)*. The incubation was continued for a further 10 min and then terminated by the addition of 5 ml ice-cold buffer to each incubation flask, the slices then being transferred to fresh ice-cold buffer. To obtain values for uptake at 0°C, parts of the slices from each region and each control and experimental animal were incubated as above, but the temperature was kept at 0°C. In the ^{3}H 5-HT uptake measurements, both total tritium and unchanged ^{3}H-5-HT were measured according to the procedure described by Björklund, Nobin and Stenevi (1973 a). The values for unchanged ^{3}H 5-HT have been corrected for recovery, which was about 45 %. In the ^{3}H NA uptake measurements, the slices were washed for 5 min in fresh bufffer at +37°C and then dissolved in 0.5 ml Soluene 100 (Packard Instr. Co.) and subjected to liquid scintillation counting. It has previously been established that unchanged NA constitutes between 85 and 100 % of the total radioactivity measured in this way (Björklund, Nobin and Stenevi 1973 a).

In the scintillation counting the counting efficiency was calculated by means of internal standards.

Fluorescence histochemistry of intraneuronal IA and CA was performed according to the Falck-Hillarp method (for details on the technique, see Björklund, Falck and Owman 1972). Control and 5,7-DHT treated specimens were processed in parallel. Some of the animals used for fluorescence histochemistry were treated with the MAO-inhibitor nialamide (300—500 mg/kg, 4—6 hrs before killing). The material used for fluorescence histochemistry comprised rats treated with low (10—75 µg) or high (100—200 µg) doses of 5,7-DHT, and included several time points between 45 min and 16 days after injection.

RESULTS

5,7-DHT was administered in doses of 10, 25, 50, 75, 100 or 200 µg (calculated as the base) into the lateral ventricle. The lower doses were well tolerated, whereas the higher ones induced severe convulsions within a few minutes after the injection. These convulsions were totally prevented by the injection of nembutal (sodium pentobarbitone, 40 mg/kg i.p.) shortly after the drug administration. With this procedure, which was regularly used with doses of 75 µg 5,7-DHT or higher, almost all animals survived the first month.

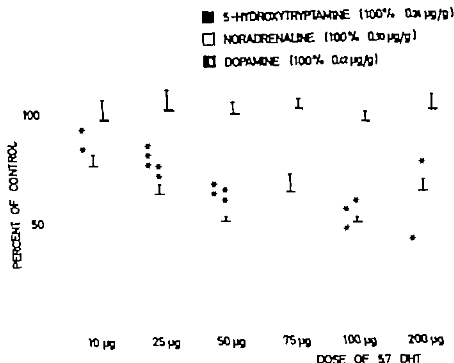


Fig. 1 Effect of six different doses of 5,7 DHT on whole brain 5-HT, NA and DA concentrations, 10 days after intraventricular injection. The bars give means ± S.E.M. of four determinations. Differences from control values $*=0.05>p>0.01$; $**=0.01>p>0.001$; $***=p<0.001$ Student's t-test.

Some of the animals treated with 200 μg 5,7 DHT were allowed to survive longer and within this group about half of the animals died unexpectedly and the remaining ones were therefore killed after 45 days.

As after treatment with 5,6-DHT the animals lost weight (about 10—15 %) after the higher doses of 5,7 DHT. The injected animals exhibited bizarre social behavior and were hyperexcitable (see Baumgarten and Lachenmayer 1972); this condition lasted until they were killed.

Effects of 5,7 DHT on brain and spinal cord monoamine content

Previous investigations on the effects of 5,6-DHT on CNS serotonin neurons showed minimum levels of 5-HT in several CNS regions at 10 days after its intraventricular injection (Baumgarten *et al.* 1971). This time was therefore chosen for an evaluation of the dose response effects of 5,7 DHT on the monoamine concentrations in whole brain (5-HT, NA and DA) and spinal cord (5-HT and NA). Fig. 1 shows that, at 10 days, there was a significant drop in both 5-HT and NA levels in whole brain, even with the lowest amount of 5,7 DHT injected (10 μg free base). With increasing doses the brain NA and 5-HT levels were equally reduced up to 50 μg, a dose that resulted in about 50 % depletion of both amines. At higher doses of 5,7 DHT 75—200

slices (3 mm in diameter and 0.5—1.0 mm thick) prepared from cortex, hypothalamus and thoracic segments of the spinal cord according to Hamberger (1967) and Sachs and Jonsson (1972). The slices were incubated as described by Björklund, Nobin and Stenevi (1973 a); the procedure being as follows: After preincubation for 10 min at +37°C in a Krebs-Ringer bicarbonate buffer (pH 7.4, saturated with 95 % O_2 and 5 % CO_2, and containing 1.8 g/l glucose) ³H 5-HT (³H 5-HT creatinine sulphate, 6.8 Ci/mmole, gen. labelled; Radiochemical Centre, Amersham, England) or ³H NA (L-NA 7-³H, 6.4 Ci/mmole; New England Nuclear, Frankfurt, G.F.R.) was added to a final concentration of 0.5×10^{-7} M (³H 5-HT) or 10^{-7} M (³H NA). The incubation was continued for a further 10 min and then terminated by the addition of 5 ml ice-cold buffer to each incubation flask, the slices then being transferred to fresh ice-cold buffer. To obtain values for uptake at 0°C, parts of the slices from each region and each control and experimental animal were incubated as above, but the temperature was kept at 0°C. In the ³H 5-HT uptake measurements, both total tritium and unchanged ³H 5-HT were measured according to the procedure described by Björklund, Nobin and Stenevi (1973 a). The values for unchanged ³H 5-HT have been corrected for recovery which was about 45 %. In the ³H NA uptake measurements the slices were washed for 5 min in fresh buffer at +37°C and then dissolved in 0.5 ml Soluene 100 (Packard Instr. Co.) and subjected to liquid scintillation counting. It has previously been established that unchanged NA constitutes between 85 and 100 % of the total radioactivity measured in this way (Björklund, Nobin and Stenevi 1973 a).

In the scintillation counting the counting efficiency was calculated by means of internal standards.

Fluorescence histochemistry of intraneuronal IA and CA was performed according to the Falck-Hillarp method (for details on the technique, see Björklund, Falck and Owman 1972). Control and 5,7-DHT treated specimens were processed in parallel. Some of the animals used for fluorescence histochemistry were treated with the MAO-inhibitor nialamide (300—500 mg/kg 4—6 hrs before killing). The material used for fluorescence histochemistry comprised rats treated with low (10—75 µg) or high (100—200 µg) doses of 5,7-DHT and included several time points between 45 min and 16 days after injection.

RESULTS

5,7-DHT was administered in doses of 10, 25, 50, 75, 100 or 200 µg (calculated as the base) into the lateral ventricle. The lower doses were well tolerated whereas the higher ones induced severe convulsions within a few minutes after the injection. These convulsions were totally prevented by the injection of nembutal (sodium pentobarbitone, 40 mg/kg i.p.) shortly after the drug administration. With this procedure, which was regularly used with doses of 75 µg 5,7-DHT or higher, almost all animals survived the first month.

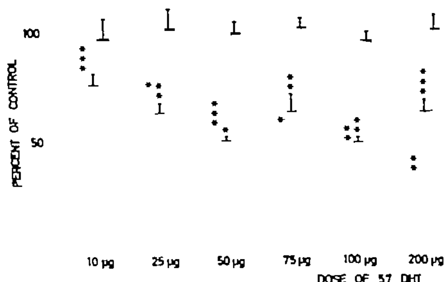


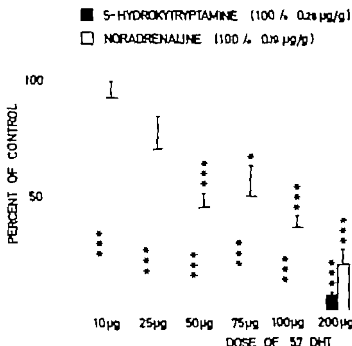
Fig 1 Effect of six different doses of 5 7 DHT on whole brain 5-HT NA and DA concentrations, 10 days after intraventricular injection. The bars give means ± S.E.M of four determinations. Differences from control values: $=0.05>p>0.01$ $=0.01>p>0.001$ $***=p<0.001$ Student' t test.

Some of the animals treated with 200 µg 5 7 DHT were allowed to survive longer and within this group about half of the animals died unexpectedly and the remaining ones were therefore killed after 45 days.

As after treatment with 5 6-DHT the animals lost weight (about 10—15 %) after the higher doses of 5 7 DHT The injected animals exhibited bizarre social behavior and were hyperexcitable (see Baumgarten and Lachenmayer 1972) this condition lasted until they were killed.

Effects of 5,7 DHT on brain and spinal cord monoamine content

Previous investigations on the effects of 5,6-DHT on CNS serotonin neurons showed minimum levels of 5-HT in several CNS regions at 10 days after its intraventricular injection (Baumgarten *et al.* 1971) This time was therefore chosen for an evaulation of the dose response effects of 5 7 DHT on the monoamine concentrations in whole brain (5-HT NA and DA) and spinal cord (5-HT and NA) Fig 1 shows that, at 10 days, there was a significant drop in both 5-HT and NA levels in whole brain, even with the lowest amount of 5 7 DHT injected (10 µg free base) With increasing doses the brain NA and 5-HT levels were equally reduced up to 50 µg, a dose that resulted in about 50 % depletion of both amines. At higher doses of 5 7 DHT 75—200



F g 2 Effect of six different doses of 5 7 DHT on spinal cord 5-HT and NA concentrations, 10 days afte intraventricular injection. The bars give means ± S E.M of four determinations. Differences from control values =0 05>p>0 01 **=0 01>p>0.001 =p<0.001 Student s t test.

µg the 5-HT concentration was further reduced (up to 75 % depletion after 200 µg) whereas no further decrease was registered in case of NA. In sharp contrast to the marked effect of 5 7 DHT on the NA concentration, the brain DA concentration was unaffected at 10 days after all doses tested (Fig 1)

In the spinal cord (Fig 2) a near maximal depletion of serotonin (about 85 % reduction) occurred already after 10 µg 5 7 DHT After this lowest dose there was no significant effect on the NA concentration However with increasing dose of 5 7 DHT the NA concentration of the spinal cord was progressively reduced after 200 µg the NA concentration was approximately 15 % of control, the reading values was, however at the limit of detectability (Fig 2) At all doses tested spinal cord 5-HT concentrations were more reduced than those of NA. As 10 µg 5 7 DHT provoked an almost maxi mal depletion of serotonin in the spinal cord but no significant changes in the NA concentration the specificity of action of 5 7 DHT on serotonin neurons in the spinal cord was greatest at this low dose

That the percentage reduction of 5-HT was not the same in all brain regions is seen in Figs. 3 A– D At 10 days after 200 µg 5 7 DHT the reduction in 5-HT varied from about 55 % in the pons + medulla oblongata preparation to about 85 % in the forebrain rest preparation and the septum. The depletion (at 10 days) was higher in the ventricle near brain regions (septum, striatum

hypothalamus) and lower in the regions comprising most of the 5-HT cell bodies (mesencephalon and pons+medulla oblongata)

The time course of effects of a single high dose of 5 7 DHT (200 µg) on CNS serotonin concentrations was evaluated on individual brain regions and on the spinal cord, and the results are summarized in Figs. 1 A—D Similar to what has been found after 5 6-DHT treatment (Baumgarten *et al* 1971) different patterns of depletion and recovery of serotonin were registered in different CNS regions. In the spinal cord (Fig 3 A) the striatum (Fig 3 C) and the forebrain rest preparation (Fig 3 D) an initial rapid depletion of about 35—60 % of the 5-HT concentration seen at 3 hrs after drug injection, was followed by a more protracted and gradual decline giving minimum levels (10—25 % of control) at 10 days. The concentrations then remained unchanged for at least another 20 days. A slight, statistically not significant recovery was noticed in both spinal cord and the striatum between 1 month and 45 days. In remaining brain regions — septum, hypothalamus, mesencephalon and pons+medulla oblongata — the acute 5 7 DHT depletion, registered at 3 hrs or 1 day was more pronounced (between 65 and 80 %) within the subsequent 3 or 4 days, there was a variable, partial, recovery of the 5-HT concentrations. This recovery was most pronounced in mesencephalon and pons+medulla oblongata, which are the regions rich in serotonin-containing cell bodies in these regions the recoveries between 3 hrs and 4 days were highly significant ($p<0.001$) In pons+medulla oblongata, mesencephalon, hypothalamus and septum there was, in addition, a tendency to a late slow recovery of the 5-HT concentrations between 10 and 45 days.

Whereas the brain DA levels were only little affected at all time points up to 10 days (Fig 4) 200 µg of 5 7 DHT caused a strong and rapid depletion of NA in brain (Fig 4) and in spinal cord (Fig 5) and at 3 hrs after injection the NA concentrations were about 25 % of control. By 4 days after injection the NA levels had recovered partially being 45 % of control in the brain and 70 % of control in the spinal cord. These recoveries between 3 hrs and 4 days were statistically significant ($0.001<p<0.01$ in both brain and spinal cord) Interestingly in the spinal cord (but not in the brain) there was a second phase of NA depletion between 4 and 10 days, from about 70 % of control at 4 days to about 15 % at 10 days (difference between 4-day and 10-day values $p<0.001$)

The reduction in brain and spinal cord NA remained up to the 45th day In fact, there was a tendency for a further reduction in brain NA concentration between 10 and 45 days (difference between 10-day and 45-day values $0.01<p<0.05$) In contrast, the whole brain DA concentration was increased at 45 days (135 % of control) this increase was not statistically significant, however

Effects of 5,7 DHT on ^{3}H-5-HT and ^{3}H NA uptake by slices of brain and spinal cord in vit o

The effect of one injection of 200 µg 5 7 DHT on the capacity of thin slices

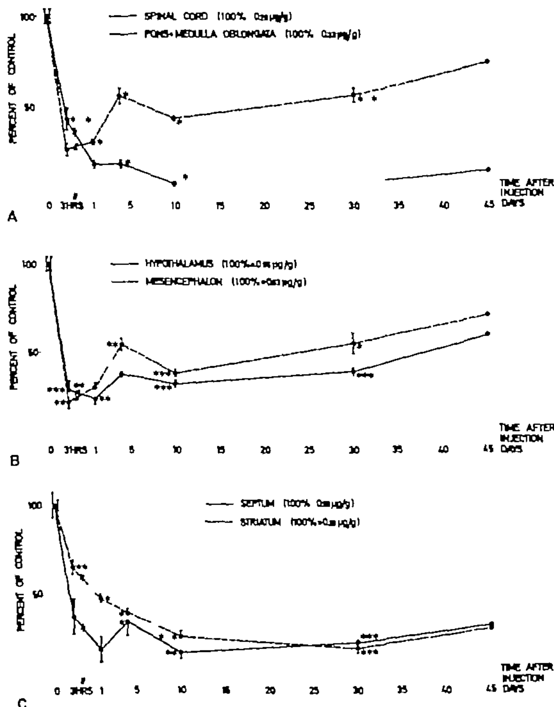
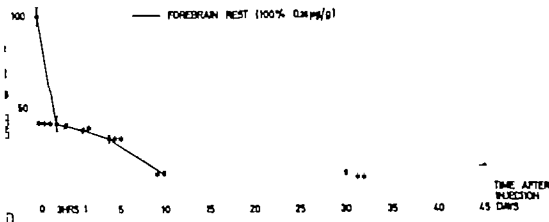


Fig. 3 A—D. Time course of effect of one single intraventricular injection of 200 μg 5,7-DHT on the 5-HT concentration in seven brain regions. Preparations from four or five rats were pooled for each determination. Each value except the 45-day values is



the mean ± S.E.M. of four to eight determinations. The 45-day values re the mean of two determinations. Differences from control alues. =0.05>p>0 01 **=0.01>p>0.001 ***=p<0.001 Student t test.

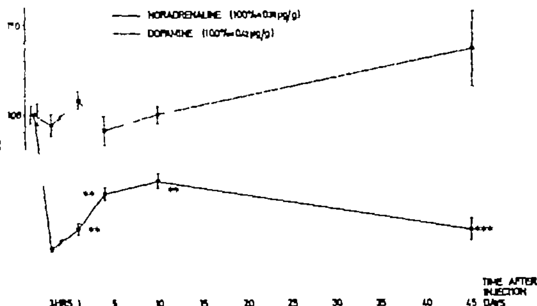
from cortex, hypothalamus and spinal cord to accumulate tritiated 5-HT or NA during a short (10 min) incubation in vitro was studied at 9—11 days after injection (Fig 6) The 5 7 DHT treatment caused a strong and statistically highly significant reduction in ^{3}H 5-HT and ^{3}H NA uptake in all three regions. Regarding the ^{3}H 5-HT uptake, the reduction was seen both in the measurements of unmetabolized 5-HT (hatched bars in Fig 6) and in the measurements of total radioactivity corresponding to 5-HT plus metabolites (open bars in Fig 6) The reduction in the uptake of unmetabolized ^{3}H 5-HT was between 85 and 90 % in all three regions, whereas the reduction in ^{3}H NA uptake was less (40—57 %) These effects on the ^{3}H 5-HT and ^{3}H-NA uptake are quite comparable to the reductions registered in the levels of endogenous 5-HT and NA at 10 days after 200 µg 5 7 DHT (see above, Figs. 3 4 and 5)

The percentage unmetabolized ^{3}H 5-HT of the total accumulated tritium in the slices was less (about 15—25 %) in the 5 7 DHT treated animals than in the control animals (about 40—55 %) This signifies that, due to the strong inhibition of the neuronal uptake, the accumulated 5-HT was more rapidly metabolized in the 5 7 DHT treated rats. As a consequence, the reduction in the uptake of unmetabolized ^{3}H-5-HT was greater than that recorded in the total accumulated tritium (Fig 6)

Preliminary fluorescence histochemical observations in 5,7 DHT-treated rats

Observations were made in rats after survival times varying from 45 min to 16 days, some of the long term animals being treated with the MAO-inhibitor nialamide (300—500 mg/kg 4—6 hrs before killing) to facilitate the visualization of IA-containing terminals.

At 45 min after the 5 7 DHT injection a diffuse, bright yellow-green formaldehyde induced fluorescence occurred in the periventricular as well



4 Time course of effect of one single intraventricular Injection of 200 µg 5,7 DHT hole brain NA and DA concentrations. Each point gives the mean ± S.E.M. of to five determinations. Differences from control values *=0.05>p>0 01 **=0.01>p>0.001 ***=p<0.001 Student' t test.

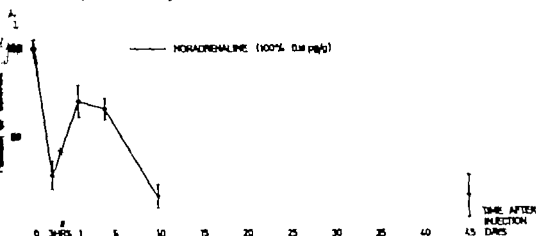


Fig 5 Time course of effect of one small intraventricular injection of 200 µg 5 7 DHT on spinal cord NA concentration. Each point gives the mean ± S.E.M. of three to five determinations. Differences from control values =0.05>p>0.01 **=0.01>p>0.001 =p<0.001 Student t test.

as in other surface-bordering parts of the brain. The colour of this fluorescence was clearly different from that of the CA or the 5-HT fluorophores, but similar to that of the fluorophore of authentic 5 7 DHT in models (unpublished observations) The yellow-green fluorescence — thus probably identical to the injected 5 7 DHT — was partly concentrated in structures resembling vari-

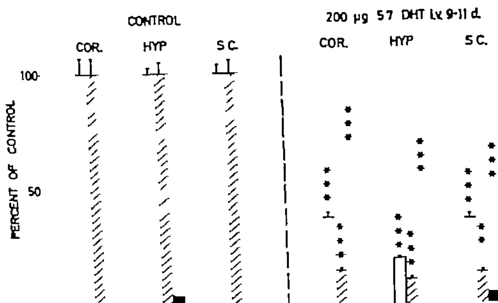


Fig 6 Effect of one injection of 200 μg of 5 7 DHT on (H)5-HT and (³H)NA ptake by brain and spinal cord slices in vitro, 9—11 days fter i jection. Slices were obtained from cortex (COR.) hypothalamus (HYP) and spinal cord (S.C.) and the alues are expressed as percent of untreated controls, and are give as means ± S E.M of 6—15 determinations. The values represent activ uptak calculated as the uptake measured t +37 C minus the uptake measured t 0 C. Differences from control values: *=0.05>p>0.01 **=0.01>p>0.001 ***=p<0.001 Student t-test.

cose monoamine axons and axon terminals. Some of these fibres — e.g in the suprachiasmatic nucleus, the subcommisural organ and the neuropil under neath the ependyma of the fourth ventricle — were probably identical to IA-containing fibres, whereas others — e.g in the periventricular hypothala mus and septum — could represent NA-containing ones. In addition, fluorescence occurred in varicose fibres within the medial forebrain bundle and in the medulla oblongata, close to the ventral surface, probably indicating an uptake of 5 7 DHT into the ascending and descending non terminal monoamine axon bundles.

The 5 7 DHT induced yellow-green fluorescence was observable up to at most, 8—12 hrs after injection thereafter the yellow fluorescent IA-containing fibres that normally are present in ventricle near regions (e.g the supra chiasmatic nuclei and the subcommisural organ) could no longer be demon strated Higher doses of 5 7 DHT (100—200 μg) caused a rapid disappearance of yellow fluorescent IA-containing fibres from ventricle near regions and partly also from ventricle-distant structures, such as the globus pallidus, the

Fig. 4. Time course of effect of one single intraventricular injection of 200 µg 5,7 DHT on whole brain NA and DA concentrations. Each point gives the mean ± S.E.M. of three to five determinations. Differences from control values * = 0.05 > p > 0.01 ** = 0.01 > p > 0.001 *** = p < 0.001 Student's t-test.

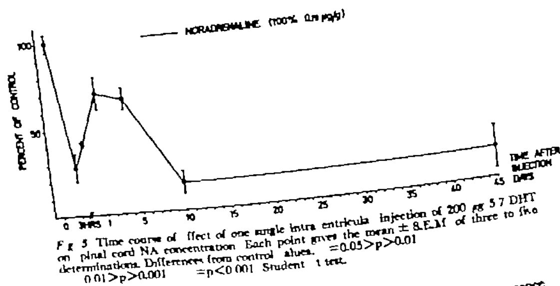


Fig. 5. Time course of effect of one single intraventricular injection of 200 µg 5,7 DHT on spinal cord NA concentration. Each point gives the mean ± S.E.M. of three to five determinations. Differences from control values. * = 0.05 > p > 0.01 ** = 0.01 > p > 0.001 *** = p < 0.001 Student's t test.

in other surface-bordering part of the brain. The colour of this fluorescence was clearly different from that of the CA or the 5-HT fluorophores, but similar to that of the fluorophore of authentic 5,7 DHT in models (unpublished observations). The yellow-green fluorescence — this is probably identical to the injected 5,7 DHT — was partly concentrated in structures resembling vari

In contrast to 5,6-DHT 5,7-DHT had pronounced and persisting effects also on the central NA neurons. Thus, 200 µg 5,7-DHT caused a 40—85 % reduction in brain and spinal cord NA levels that persisted for at least 45 days, and there was a 40—57 % reduction in the ^{3}H-NA uptake capacity of slices from cortex, hypothalamus and spinal cord at 9—11 days after injection, suggesting that 5,7-DHT induces degeneration also in NA neurons. This is further supported by the fluorescence microscopical observations of a long-term disappearance of fluorescent, presumed NA terminals, and of signs of extensive axonal damage in NA axon bundles. It is notable that, following doses up to 50 µg the degree of depletion at 10 days was similar for 5-HT and NA in the brain. After higher doses (75—200 µg) 5-HT was further reduced (up to 75 % after 200 µg) whereas NA remained at about 50 % of control. It is remarkable that even after 200 µg of 5,7-DHT the DA neurons appeared largely unaffected by the treatment.

Studies on the impairment of ^{3}H-5-HT and ^{3}H-NA uptake into cortical slices *in vitro* induced by a preceding exposure of the slices to 5,7-DHT (Björklund, Baumgarten, Nobin and Schlossberger in preparation) have provided evidence that 5,7-DHT exerts direct toxic effects on both IA and CA axons, but that the effect on the IA axons is considerably stronger. This has also been shown in studies on the effects of localized, intracerebral or intraspinal injections of 5,7-DHT (Björklund, Nobin and Stenevi 1973 c) demonstrating that IA axons are more susceptible than CA axons to 5,7-DHT, but that both DA and NA fibres are partially damaged. The fact that after intraventricular injections this higher susceptibility of the IA neurons to 5,7-DHT is revealed in the brain only after the highest doses is most probably due to the difference in topography of the different monoamine neuron systems, and this readily explains also why the IA neurons innervating the spinal cord are much more susceptible than those of the brain to neurotoxic drugs administered via the cerebrospinal fluid (see Nobin *et al.* 1973). In the brain, extensive NA-containing terminal systems are located close to the ventricles and one of the major ascending NA fibre bundles (the so-called dorsal CA bundle) is situated close to the aqueduct (see Ungerstedt 1971). This is in contrast to the IA-containing fibre systems which are more evenly distributed in the brain. In spinal cord the situation is rather the reverse here, the descending IA axon are located very superficially in the white matter and are thus easily reached from the cerebrospinal fluid (Nobin *et al.* 1973).

At the same dose level, e.g. 50 or 75 µg, 5,6-DHT and 5,7-DHT have roughly the same depletory effect on the whole brain or spinal cord 5-HT concentrations (compare with data from Baumgarten *et al.* 1971, Da Prada, Carruba, O'Brien, Saner and Pletscher 1972, Baumgarten, Björklund and Nobin, unpublished observations). This is interesting in view of the uptake affinity data of Horn *et al.* (1973). According to their measurements, the affinity of 5,7-DHT for the 5-HT uptake mechanism (measured as the concentration of the drug causing 50 % inhibition of the uptake and retention of ^{3}H-5-HT by synaptosomes from rat hypothalamus) was about 6 times less than

that of 5,6-DHT. Thus, while 5,6-DHT has a very high affinity for the 5-HT uptake mechanism (comparable to 5-HT itself) and a much lower affinity for the NA or DA uptake mechanisms (Baumgarten *et al.* 1972 b), 5,7-DHT has, in fact, a higher affinity for the NA uptake mechanism than for the 5-HT mechanism (Horn *et al.* 1973). Since the selective neurotoxic action of the dihydroxylated tryptamines on the monoamine neurons most probably depends on their uptake and accumulation within the neurons, it is notable that 5,7-DHT is as potent on the serotonin neurons as 5,6-DHT despite its considerably lower affinity for the 5-HT uptake system. This points to a higher general neurotoxic potency of 5,7-DHT, which at least partly could be due to 5,7-DHT not being as sensitive to oxidation at physiological pH as 5,6-DHT which to some extent precipitates in the form of a brownish pigment on the ventricle walls. The higher stability of 5,7-DHT will most likely provide the substance with better diffusion properties and allow it to reach deeper into the brain tissue.

Although the neurotoxic effects of 5,7-DHT and 5,6-DHT with respect to the serotonin neurons appears to be similar, 5,7-DHT has a clear advantage over 5,6-DHT in being markedly less toxic. This allowed the administration of much higher doses of 5,7-DHT into the cerebrospinal fluid and made possible a more pronounced denervation of several brain regions. With 5,6-DHT a comparable degree of denervation can be achieved in diencephalic and telencephalic areas only after local injections close to the ascending IA fibre systems (Björklund *et al.* 1973 c). On the other hand, the selectivity of 5,7-DHT is less, and it is clear that 5,7-DHT affects the NA neurons to a considerably greater extent than 5,6-DHT does.

Two different patterns could be distinguished in the timecourse of effects of 5,7-DHT on the 5-HT content in different CNS regions. (1) In ventricle near regions (septum and hypothalamus) as well as in the cell body rich parts (mesencephalon and pons+medulla oblongata) a profound reduction in 5-HT (up to 80 %) was registered by 3—24 hrs. Following a transient recovery phase between 1 and 4 days after injection, the 5-HT levels declined to near minimum values (55—85 % reduction) at 10 days. (2) The regions comprising tissue more distant from the ventricles — the spinal cord, the forebrain rest sample and the striatum — an initial, rapid depletion of about 35—60 % of the 5-HT concentration, seen at 3 hrs after injection, was followed by a more protracted and gradual decline giving minimum levels (10—25 % of control) at 10 days. The patterns of 5-HT depletion should be possible to interpret as a sequence of different events that occur to a different extent in different regions, and that cannot be clearly separated from one another: (a) an initial rapid displacement phase, followed by (b) a recovery of some of the depleted terminals, and by (c) an acute degeneration of other terminals; these events are followed by (d) a protracted phase of anterograde degeneration of terminals that were not initially reached by the drug.

The several events induced by 5,7-DHT in the central serotonin neurons are similar to those previously described in detail by Baumgarten *et al.* (1971)

Nobin *et al.* (1973) and Björklund *et al.* (1973 a, b c) after 5 6-DHT treatment. However because 5 7 DHT was administered in higher doses than 5 6-DHT the magnitude of both the acute (3—24 hrs after injection) and the long term effects was greater after 5 7 DHT (200 µg) than after 5 6-DHT (75 µg)

ACKNOWLEDGEMENTS

The authors are indebted to Lilian Bengtsson, Ann-Marie Olsson, Kerstin Forel *tr̃en* and Rolf Frank for their skilful assistance. 5 7-dihydroxytryptamine was kindly supplied by Dr. A. Manian, N.I.M.H., Bethesda/Md., USA.

The study was supported by grants from Svenska Livförsäkringsbolags Nämnd *för* Medicinsk Forskning, from the National Institutes of Health USPHS (06701-07) *and* from the Deutsche Forschungsgemeinschaft, and was carried out within a research organization sponsored by the Swedish Medical Research Council (grants No. 04X-712 04X 56, and 04X-3874)

BAUMGARTEN, H G A. BJÖRKLUND, A. HOLSTEIN and A. NOBIN Chemical degeneration of indolamine axons in rat brain by 5 6-dihydroxytryptamine, an ultra structural study *Z Zellforsch.* 1972 a. *129* 256—271

BAUMGARTEN H. G., A. BJÖRKLUND L. LACHENMAYER, A. NOBIN and U STENEVI Long lasting selective depletion of brain serotonin by 5 6-dihydroxytryptamine. *Acta physiol. scand* 1971 Suppl. *373* 1—16.

BAUMGARTEN, H. G., K. D EVETTS R. B. HOLMAN L. L. IVERSEN M. VOGT and G WILSON Effects of 5,6-dihydroxytryptamine on monoaminergic neurones in the central nervous system of the rat. *J Ne ochem* 1972 b. *19* 1587—1597

BAUMGARTEN H. G and L. LACHENMAYER, 5 7 Dihydroxytryptamine Improvement in chemical lesioning of indoleamine neurons in the mammalian brain. *Z Zellforsch* 1972. *135* 399—414

BAUMGARTEN H. G., L. LACHENMAYER and H. G SCHLOSSBERGER, Evidence for a degeneration of indolamine-containing nerve terminals in rat brain, induced by 5 6-dihydroxytryptamine. *Z. Zellforsch.* 1972 c. *125*. 553—569.

BAUMGARTEN H. G S. J VICTOR and W LOVENBERG Effect of intraventricular injection of 5 7-dihydroxytryptamine on regional tryptophan hydroxylase of rat brain. *J Ne rochem.* In press. 1973

BERTLER, A., Effect of reserpine on the storage of catecholamines in brain and other tissues. *A ta physiol sc nd.* 1961 *51* 75—85

BERTLER, A., A. CARLSSON E. ROSENGREN and B. WALDECK A method for the fluorimetric determinations of adrenaline noradrenaline, and dopamine in tissues. *K gl. Fysiogr Sällsk. Lund Förh* 1958 *28* 121—123

BJÖRKLUND A., H. G BAUMGARTEN A. NOBIN and H G SCHLOSSBERGER, In preparation. 1973 d.

BJÖRKLUND A., B. FALCK and CH. OWMAN Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *M thods I I vestigative nd Diagnostic Endocrinology* (Ed S. A. Berson), Vol. 1 *The Thyroid nd Biogenic Amines* (Eds. J E. Rall and I J Kopin) North-Holland Publ. Comp., Amsterdam 1972 a. pp. 318—368.

BJÖRKLUND A., A. NOBIN and U STENEVI Effects of 5 6-dihydroxytryptamine on nerve terminal serotonin and serotonin uptake in the rat brain. *Brain. Res.* 1973 a. *53* 117—127

BJÖRKLUND A., A. NOBIN and U STENEVI Regeneration of central serotonin neurons after axonal degeneration induced by 5,6-dihydroxytryptamine. *Bra Res.* 1973 b. *50* 214—220

BJÖRKLUND A., A. NOBIN and U STENEVI The use of neurotoxic dihydroxytryptamines as tools for morphological studies on central indolamine neurons. *Comm. Dept A at Lu d* 1973 c. *3* 1—26

DA PRADA M M. CARRUBA, R. A. O'BRIEN A. SANER and A. PLETSCHER, The effect of 5,6-dihydroxytryptamine on sexual behaviour of male rats. *Europ. J Pharm col* 1972. *19* 288—290

DALY, J K. FUXE and C JONSSON Effects of intracerebral injections of 5,6-dihydroxytryptamine on central monoamine neurons. Evidence for selective degeneration of central 5-hydroxytryptamine neurons. *Bra Res.* 1973 *49* 476—482

HAMBERGER, B., Reserpine-resistant uptake of catecholamines in isolated tissues of the rat. *Acta physiol. sc nd* 1967 Suppl. *295* 1—47

HORN A. S., H. G. BAUMGARTEN H. G SCHLOSSBERGER Inhibition of the uptake of 5-hydroxytryptamine, noradrenaline and dopamine into rat brain homogenates by various hydroxylated tryptamines. *J Neurochem* In press 1973

HÄGGENDAL, J An improved method for fluorometric determination of small amounts of adrenaline and noradrenaline in plasma and tissues. *Acta physiol. scand.* 1963 *59* 242—254

NOBIN A., H. G BAUMGARTEN A BJÖRKLUND, L. LACHENMAYER and U STENEVI Axonal degeneration and regeneration of the bulbo-spinal indolamine neurons after 5,6-dihydroxytryptamine treatment. *B ain Res.* 1973 *56* 1—24

SACHS CH. and G JONSSON Degeneration of central and peripheral noradrenaline neurons produced by 6-hydroxy-DOPA *J Neurochem.* 1972. *19* 1561—1575.

UNGERSTEDT U Histochemical studies on the effects of intracerebral and intraventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In: *6-Hydroxydopamine and catecholamine neurons* (Eds T Malmfors and H Thoenen) North-Holland, Amsterdam-London 1971 pp. 101—127

REFERENCES

BAUMGARTEN H G A. BJÖRKLUND, A. HOLSTEIN and A. NOBIN Chemical degeneration of indolamine axons in rat brain by 5 6-dihydroxytryptamine, an ultrastructural study *Z. Zellforsch.* 1972 a. *129* 256—271

BAUMGARTEN H. G A. BJÖRKLUND L. LACHENMAYER, A. NOBIN and U STENEVI Long lasting selective depletion of brain serotonin by 5,6-dihydroxytryptamine. *Acta physiol. scand* 1971 Suppl. *373* 1—16.

BAUMGARTEN H. G., K. D. EVETTS, R. B. HOLMAN L. L. IVERSEN M VOGT and G WILSON Effects of 5 6-dihydroxytryptamine on monoaminergic neurones in the central nervous system of the rat. *J Neurochem.* 1972 b. *19* 1587—1597

BAUMGARTEN H. G and L. LACHENMAYER, 5 7 Dihydroxytryptamine Improvement in chemical lesioning of indoleamine neurons in the mammalian brain. *Z. Zellforsch.* 197_ *135* 399—414

BAUMGARTEN H. G., L. LACHENMAYER and H. G. SCHLOSSBERGER, Evidence for degeneration of indolamine-containing nerve terminals in rat brain, induced by 5,6-dihydroxytryptamine. *Z Zellforsch.* 1972 c. *125* 553—569.

BAUMGARTEN H. G., S. J VICTOR and W LOVENBERG Effect of intraventricular injection of 5 7-dihydroxytryptamine on regional tryptophan hydroxylase of rat brain. *J Neurochem* In press 1973

BERTLER, A., Effect of reserpine on the torage of catecholamines in brain and othe tissues *Act physiol. scand* 1961 *51* 75—83

BERTLER, A A. CARLSSON E. ROSENGREN and B. WALDECK, A method for the fluorimetric determinations of adrenaline, noradrenaline, and dopamine in tissues. K gl. Fysiogr Sällsk. Lund F8 k 1958 *28* 121 —123

BJÖRKLUND A., H G BAUMGARTEN A. NOBIN and H. G SCHLOSSBERGER, In preparation. 1973 d.

BJÖRKLUND A., B FALCK and CH OWMAN Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In *Methods I I vestigative and Diagnostic Endocri ology* (Ed S A. Berson), Vol. 1: *The Thyroid and Biog nic Am ne* (Eds. J E. Rall and I J Kopin) North-Holland Publ. Comp., Amsterdam 1972 a. pp. 318—368.

BJÖRKLUND A., A. NOBIN and U STENEVI, Effects of 5 6-dihydroxytryptamine on nerve terminal serotonin and serotonin uptake in the rat brain. *Brain. Res.* 1973 a. *53* 117—127

BJÖRKLUND A., A. NOBIN and U STENEVI, Regeneration of central serotonin neurons after axonal degeneration induced by 5,6-dihydroxytryptamine. *Bra Res* 1973 b *50* 214—220

BJÖRKLUND A., A. NOBIN and U STENEVI The use of neurotoxic dihydroxytryptamines as tools for morphological studies on central indolamine neurons. *Comm Dept A at Lund* 1973 c. *3* 1—26.

DA PRADA, M., M. CARRUBA, R. A. O'BRIEN A. SANER and A. PLETSCHER, The effect of 5 6-dihydroxytryptamine on sexual behaviour of male rats. *E op. J Pharmacol.* 1972. *19* 288—290.

DALY, J K. FUXE and G. JONSSON Effects of intracerebral injections of 5,6-dihydroxytryptamine on central monoamine neurons. Evidence for selective degeneration of central 5-hydroxytryptamine neurons. *B ai Res.* 1973. *49* 476—482.

HAMBERGER, B., Reserpine-resistant uptake of catecholamines in isolated tissues of the rat. *Acta physiol. scand* 1967 Suppl. *295* 1—47

HORN A. S., H. G BAUMGARTEN H. G. SCHLOSSBERGER, Inhibition of the uptake of 5-hydroxytryptamine, noradrenaline and dopamine into rat brain homogenates by various hydroxylated tryptamines. *J Neurochem.* In press 1973.

HÄGGENDAL, J An improved method for fluorometric determination of small amounts of adrenaline and noradrenaline in plasma and tissues. *Act physiol. sc d* 1963 *59* 242—254

NOBIN A., H. G BAUMGARTEN A. BJÖRKLUND, L. LACHENMAYER and U STENEVI Axonal degeneration and regeneration of the bulbo-spinal indolamine neurons after 5,6-dihydroxytryptamine treatment. *Brain Res.* 1973 56 1—24

SACHS, CH. and G. JONSSON Degeneration of central and peripheral noradrenaline neurons produced by 6-hydroxy-DOPA *J Neurochem.* 1972. *19* 1561—1575.

UNGERSTEDT U., Histochemical studies on the effects of intracerebral and intra ventricular injections of 6-hydroxydopamine on monoamine neurons in the rat brain. In *6-Hydroxydopamine and catecholamine neurons* (Eds T Malmfors and H Thoenen) North-Holland, Amsterdam-London 1971 pp. 101—127

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 392

RECURRENT CONTROL FROM MOTOR AXON COLLATERALS OF Ia INHIBITORY PATHWAYS IN THE SPINAL CORD OF THE CAT

BY

SIVERT LINDSTRÖM

GÖTEBORG 1973

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 392

From the Department of Physiology University of Göteborg, Sweden

RECURRENT CONTROL FROM MOTOR AXON COLLATERALS OF Ia INHIBITORY PATHWAYS IN THE SPINAL CORD OF THE CAT

BY

SIVERT LINDSTRÖM

GÖTEBORG 1973

CONTENTS

INTRODUCTION 5

METHODOLOGICAL CONSIDERATIONS 8

RESULTS AND COMMENTS 11

A Recurrent effects from motor axon collaterals on interneurones in Ia inhibitory pathways 11
1 To α-motoneurones 11
2 To VSCT neurones 13
3 To DSCT neurones 14
4 To segmental interneurones 14
5 To Ia primary afferents 15

B Selectivity in the recurrent control of Ia inhibitory pathways 15

C Is the Ia inhibition of VSCT neurones and α-motoneurones mediated by the same interneurones ? 17

D Recurrent inhibition of VSCT neurones from motor axon collaterals 18

E Composition of the VSCT neuronal pool with respect to monosynaptic excitation from muscle afferents 19

GENERAL DISCUSSION 22

A Comments on the functional significance of the recurrent control of the Ia inhibitory pathway to motoneurones 22

B Information transfer in the VSCT 25

SUMMARY AND CONCLUSIONS 34

ACKNOWLEDGEMENTS 35

REFERENCES 36

This thesis mainly constitutes a summary of the following articles:

I Hultborn H, E Jankowska and S Lindström: Recurrent inhibition from motor axon collaterals of transmission in the Ia inhibitory pathway to motoneurones. J Physiol (Lond) 1971 18 591–61

II Gustafsson B and S Lindström: Recurrent control from motor axon collaterals of Ia inhibitory pathways to ventral spinocerebellar tract neurones. Acta physiol scand 1973 In press

III Lindström S and E D Schomburg: Group I inhibition in Ib excited ventral spinocerebellar tract neurones. Acta physiol scand 1973 In press

IV Lindström S and E D Schomburg: Recurrent inhibition from motor axon collaterals of ventral spinocerebellar tract neurones. Acta physiol scand 1973 In press

The papers are referred to by their Roman numerals in the text

INTRODUCTION

The inhibitory pathways from large muscle spindle (Ia) afferents to motoneurones and from motor axon collaterals to motoneurones are two of the best known inhibitory pathways in the mammalian central nervous system The former was described by Lloyd (1941 1943 a b 1946 a, b) who found that the largest muscle afferents which supply monosynaptic excitation to motoneurones innervating their own and synergistic muscles also give reciprocal inhibition to motoneurones of muscles acting as antagonists at the same joint Lloyd (1943 b c) further showed that the excitatory two-neurone reflex arch from these afferents formed the basis for the stretch reflex of Liddell and Sherrington (1924) Extensor and flexor muscles acting at a given joint are thus linked by the excitatory and inhibitory reflex connecting from these afferents into a "myotatic unit" (Lloyd 1946 b) Already these early studies indicated that the afferents responsible for the effects originate from muscle spindle primary endings a view which has been fully substantiated by later experiments (cf Matthews 1972)

The reciprocal Ia inhibitory pathway was considered by Lloyd to be monosynaptic like the Ia excitatory path – thus the name direct inhibition However later experiments have demonstrated that an interneurone is interposed in the inhibitory pathway This conclusion rests on precise measurements of the latencies of the inhibitory responses in motoneurones (Eccles, Fatt and Landgren 1956 Araki Eccles and Ito 1960) and on the demonstration of spatial facilitation in the pathway (R M Eccles and Lundberg 1958 a) The interposed interneurone was initially seen largely as a device to change the excitatory action of primary afferents into inhibition (cf Eccles et al. 1956) but later investigators have stressed the integrative role of this interneurone (cf Lundberg 1970 Hultborn 1972 a, b) Several descending and segmental systems have been found to converge onto the Ia inhibitory interneurones (cf Lundberg 1970 Hultborn 1972 a b) and the first part of this study added a further one, namely the recurrent inhibitory path from motor axon collaterals through Renshaw cells

That impulses in motor axons can affect the excitability of other motoneurones was demonstrated by Renshaw (1941) who activated motor axons antidromically and observed that such stimuli caused inhibition or facilitation of other motoneurones The recurrent inhibitory pathway to motoneurones was subsequently analyzed in detail by Eccles Fatt and Koketsu (1954) who gave convincing evidence for a disynaptic action from motor axon collaterals, through a specific

inhibitory interneurone This interneurone was named the Renshaw cell by Eccles et al (1954) in honor of Renshaw who first described these cells (Renshaw 1946)

The recurrent facilitatory effects have been analyzed by Wilson and collaborators (Wilson 1959 Wilson, Diecke and Talbot 1960 Wilson and Burgess 1962 a, b) They showed that the facilitation is in fact a disinhibition, due to recurrent inhibition from motor axon collaterals through Renshaw cells of some inhibitory interneurones tonically impinging upon motoneurones This finding formed the starting point for the first part of the present study On the assumption that these recurrently inhibited interneurones were interposed as a link in some inhibitory pathway to motoneurones and that the recurrent control of any such pathway was functionally important the effect of stimulation of motor axon collaterals was tested on transmission in different segmental inhibitory reflex pathways to motoneurones As mentioned, the interneurones in the reciprocal Ia inhibitory pathway to motoneurones were found to receive recurrent inhibition from motor axon collaterals This finding has initiated a series of studies which have considerably increased our knowledge about the Ia inhibitory pathway both from a physiological and anatomical point of view (Hultborn Jankowska and Lindström 1971 a, b Hultborn, Jankowska, Lindström and Roberts 1971 Fedina and Hultborn 1972 Hultborn 1972 a, b Hultborn and Lundberg 1972 Hultborn and Santini 1972 Hultborn and Udo 1972 a b c Jankowska and Roberts 1972 a b Jankowska and Lindström 1972 Jankowska, Lundberg and Stuart 1973)

Disynaptic inhibition from Ia afferents is evoked not only in motoneurones but also in cells of origin of the ventral (Eccles Hubbard and Oscarsson 1961) and the dorsal spinocerebellar tracts (Curtis Eccles and Lundberg 1958 Eccles Oscarsson and Willis 1961) Parallel with the above studies of the recurrent control of the reciprocal Ia inhibitory pathway to motoneurones Lundberg and Weight (1971) performed experiments on the ventral spinocerebellar tract (VSCT) which led to the hypothesis that the VSCT conveys information about transmission in segmental inhibitory pathways to motoneurones (Lundberg 1971) This hypothesis should be considered in relation to the extensive convergence from different segmental and descending sources onto the interneurones in such reflex pathways (cf Lundberg 1966 1969 a, 1970 Hultborn 1972 b and Fig 1) suggesting that higher centres might need some feedback information from these interneurones in order to achieve an accurate control of movements

Central to the new VSCT hypothesis is the postulate that VSCT neurones receive collateral connexions from the last order inhibitory interneurones in reflex pathways to motoneurones (Lundberg 1971) The recurrent control of the interneurones in the reciprocal Ia inhibitory

pathway to motoneurones offered an excellent opportunity to test this postulate Accordingly the observed parallelism in the recurrent control of Ia inhibitory pathways to motoneurones and VSCT neurones provides strong indirect evidence that these two systems do receive collateral connexions from common interneurones The second part of this thesis is devoted to an evaluation of the capability of the VSCT to signal information about inhibitory pathways to motoneurones using the reciprocal Ia inhibitory pathway as a model

inhibitory interneurone This interneurone was named the Renshaw cell by Eccles et al (1964) in honor of Renshaw who first described these cells (Renshaw 1946)

The recurrent facilitatory effects have been analyzed by Wilson and collaborators (Wilson 1959 Wilson Diecke and Talbot 1960 Wilson and Burgess 1962 a b) They showed that the facilitation is in fact a disinhibition due to recurrent inhibition from motor axon collaterals through Renshaw cells of some inhibitory interneurones tonically impinging upon motoneurones This finding formed the starting point for the first part of the present study On the assumption that these recurrently inhibited interneurones were interposed as a link in some inhibitory pathway to motoneurones and that the recurrent control of any such pathway was functionally important, the effect of stimulation of motor axon collaterals was tested on transmission in different segmental inhibitory reflex pathways to motoneurones As mentioned, the interneurones in the reciprocal Ia inhibitory pathway to motoneurones were found to receive recurrent inhibition from motor axon collaterals This finding has initiated a series of studies which have considerably increased our knowledge about the Ia inhibitory pathway both from a physiological and anatomical point of view (Hultborn Jankowska and Lindström 1971 a b Hultborn, Jankowska Lindström and Roberts 1971 Fedina and Hultborn 1972 Hultborn 1972 a b Hultborn and Lundberg 1972 Hultborn and Santini 1972 Hultborn and Udo 1972 a, b c Jankowska and Roberts 1972 a, b Jankowska and Lindström 1972 Jankowska Lundberg and Stuart 1973)

Disynaptic inhibition from Ia afferents is evoked not only in moto neurones but also in cells of origin of the ventral (Eccles Hubbard and Oscarsson 1961) and the dorsal spinocerebellar tracts (Curtis Eccles and Lundberg 1958 Eccles Oscarsson and Willis 1961) Parallel with the above studies of the recurrent control of the reciprocal Ia inhibitory pathway to motoneurones Lundberg and Weight (1971) performed experiments on the ventral spinocerebellar tract (VSCT) which led to the hypothesis that the VSCT conveys information about transmission in segmental inhibitory pathways to motoneurones (Lundberg 1971) This hypothesis should be considered in relation to the extensive convergence from different segmental and descending sources onto the interneurones in such reflex pathways (cf Lundberg 1966 1969 a, 1970 Hultborn 1972 b and Fig 1) suggesting that higher centres might need some feedback information from these interneurones in order to achieve an accurate control of movements

Central to the new VSCT hypothesis is the postulate that VSCT neurones receive collateral connexions from the last order inhibitory interneurones in reflex pathways to motoneurones (Lundberg 1971) The recurrent control of the interneurones in the reciprocal Ia inhibitory

volleys do not elicit changes in the membrane conductance of the recorded cells or primary afferent depolarization this method allows conclusions to be drawn regarding the convergence on the interneurones in the studied reflex pathway An increase of test IPSPs by the conditioning volleys indicates an excitatory convergence onto the inter neurones and a decrease an inhibitory one. This indirect approach is superior to direct recordings from interneurones as a first step in analyzing interneuronal properties since individual interneurones usually cannot be identified as belonging to a defined reflex pathway (cf Lundberg 1969 a)

Electrical stimulation of peripheral nerves and ventral roots has been used throughout this study since this method allows accurate timing of the responses and controlled activation of afferents in several muscle and cutaneous nerves in the same experiment Single stimuli were used both for evoking the test responses from different types of afferents and for the conditioning stimulation of ventral roots The test IPSPs were usually submaximal for a given fibre group in order to increase the sensitivity of the testing while the conditioning ventral root volleys were maximal for α-fibres In the motoneurone experiments (paper I) only those of the ventral roots L6 – S1 which did not evoke recurrent inhibition in the recorded motoneurones were used for conditioning stimulation while all the ventral roots L6 – S1 were used in the VSCT experiments (papers II and III) since the recorded VSCT neurones did not receive recurrent inhibition from motor axons (cf however paper IV) The unconditioned and the conditioned responses were usually averaged to minimize errors due to synaptic noise and random variations in the responses

The analysis has been concerned mainly with synaptic effects from group I muscle afferents but group II and III muscle afferents and cutaneous afferents have also been stimulated The group I muscle afferents are connected with muscle spindle primary endings (Ia afferents) and with Golgi tendon organs (Ib afferents) The difference in threshold for electrical stimulation of these afferents has been utilized to differentiate between the synaptic responses evoked by them The main con clusions rest on observations of effects from nerves to thigh muscles for which the electrically elicited group I nerve volleys usually display a double configuration in triphasic recordings from the dorsal root entry zone (Bradley and Eccles 1953) It is well established that the first low threshold component in such group I volleys is mainly due to activation of Ia afferents and that the second higher threshold component largely is due to activation of Ib afferents (Bradley and Eccles 1953 Laporte and Bessou 1957 Eccles et al 1957 a c cf also Matthews 1972) Even if a certain degree of overlap exists between these two groups of afferents the main contribution to the recorded synaptic potentials from Ia or Ib

afferents can usually be determined by carefully grading the responses by varying the strength of stimulation The differentiation is considerably sharpened up by testing with the double volley technique (Bradley and Eccles 1953 Eccles Eccles and Lundberg 1957 a) which usually has been used in this study By careful grading it is often also possible to classify tentatively the effects from nerves which do not display clearcut differences in the conduction velocity of the Ia and Ib fibres (usually nerves to leg muscles) into Ia or Ib responses, since also in such cases the Ia fibres are largely recruited in the lower threshold group I range and the Ib fibres in the higher threshold group I range (Eccles et al 1957 a c cf Matthews 1972) The effects from high threshold muscle afferents (group II and III) and cutaneous afferents were confined to the flexor reflex pattern (FRA R M Eccles and Lundberg 1959 Holmqvist and Lundberg 1961) and were usually easy to differentiate from responses of group I afferents

Although synchronous electrical stimulation of peripheral nerves is a highly unnatural way of activating afferents from different types of receptors this method has proved to be a valuable tool in determining central actions of such afferents To understand the functional significance of connexions found in this way it is necessary to know when and to which degree they are utilized in transmission of adequately evoked discharges an information which cannot be obtained with the present technique However the results offer a useful basis for tentative interpretations which subsequently can be tested with other experimental approaches

ABBREVIATIONS

DSCT dorsal spinocerebellar tract; VSCT ventral spinocerebellar tract; EPSP excitatory postsynaptic potential; IPSP inhibitory postsynaptic potential FRA flexor reflex afferents

RESULTS AND COMMENTS

A Recurrent effects from motor axon collaterals on interneurones in Ia inhibitory pathways

1 To α-motoneurones Disynaptic IPSPs are evoked in α-motoneurones largely from Ia afferents in nerves to antagonist muscles (Lloyd 1946 b R M Eccles and Lundberg 1956 b) In paper I it was demonstrated that such IPSPs are effectively depressed by a preceding conditioning antidromic volley in motor axons The effect was found on Ia IPSPs in practically all tested motoneurones supplying different flexor or extensor muscles of the hindlimb and seems therefore to be a general property of Ia inhibitory pathways to hindlimb motoneurones Since the depression occurred without any concomitant conductance change in the membrane of the recorded motoneurones and without excitability changes in Ia primary afferents it was concluded that the observed effect was due to inhibitory interaction at the interneuronal level in the Ia inhibitory pathway

By varying the interval between the conditioning and the testing volleys it was found that the onset of the depression occurred at an interval indicating a disynaptic linkage from motor axons to the Ia inhibitory interneurones and that the time course of the depression corresponded to the time course of recurrent inhibition of motoneurones (Renshaw 1941 Eccles et al 1954) This and some other observations led to the conclusion that the recurrent depression of the Ia IPSPs was caused by postsynaptic inhibition of the Ia inhibitory interneurones mediated from motor axon collaterals through Renshaw cells The pathway should thus be similarly organized as the recurrent inhibitory pathway to α-motoneurones (Eccles et al 1954)

The above conclusions have been fully supported in subsequent investigations which will be briefly summarized By means of monosynaptic test reflexes (cf Fig 8 paper I) it has been found that the duration of the recurrent depression of the Ia inhibition of α-motoneurones is prolonged by intravenous administration of the anticholinesterase, eserine and decreased by the cholinergic blocking agent, dihydro-β erythroidine (Gustafsson and Lindström, unpublished) This indicates that the recurrent inhibitory pathway to the Ia inhibitory interneurones like the one to motoneurones, contains a cholinergic synapse (between motor axon collaterals and Renshaw cells; cf Eccles et al 1954)

Interneurones which fulfil all requirements of cells which should relay reciprocal Ia inhibition to motoneurones e g monosynaptic excitation from Ia afferents and disynaptic inhibition from motor axons have been found in the ventral horn just dorsomedially of the motor nuclei (Hultborn et al 1971 a) By simultaneous recording from such Ia interneurones and supposed target motoneurones Jankowska and Roberts (1972 b) were able to show that action potentials in the inter neurones were followed by monosynaptic unit IPSPs in the motoneurones thereby obtaining direct proof that these interneurones were interposed as a link in the reciprocal Ia inhibitory pathway to motoneurones The disynaptic Ia inhibitory pathway to motoneurones is thus the first inter neuronal pathway in the mammalian central nervous system which has been fully identified physiologically in all parts The interneurones in this pathway have been identified also morphologically by intracellular staining with Procion Yellow (Jankowska and Lindström 1972 1973) It should be pointed out that these interneurones are not identical with the Ia excited interneurones in the intermediate region which originally were supposed to mediate Ia reciprocal inhibition to motoneurones (Eccles et al 1956) These latter interneurones lack recurrent inhibi tion and presumably project to some other neurones than motoneurones (Hultborn et al 1971 a cf Discussion paper III)

The recurrent inhibition of α-motoneurones is supplied by α-motor axon collaterals (Eccles et al 1954) and the same is true for the recurrent depression of Ia IPSPs in motoneurones (paper I) However different motor axons are responsible for the recurrent inhibition of a given motoneurone and for the depression of Ia IPSPs in the same cell

The recurrent inhibition is supplied mainly by efferents to muscles coupled in Ia synergism while nerves to antagonist muscles are without effects (Eccles, Eccles Iggo and Ito 1961 Hultborn et al 1971 b) Since there is generally an overlap in the rostrocaudal distribution of synergistic motor nuclei the recurrent inhibition is evoked mainly from ventral roots neighbouring the one containing the axon of the recorded cell This is best illustrated with motoneurones of the antagonist muscles posterior biceps-semitendinosus (knee flexors) and quadriceps (knee extensor) which are located at two different levels in the spinal cord, posterior biceps semitendinosus motoneurones in the L7 – S1 segments and quadriceps motoneurones in the L5 and rostral L6 seg ments The former cells receive their main recurrent inhibition from the L7 and S1 ventral roots and the latter their main recurrent inhibition from the L5 and L6 ventral roots (cf paper I)

The Ia IPSPs in the posterior biceps-semitendinosus motoneurones (evoked by Ia afferents in the quadriceps nerve) are on the other hand depressed from the L5 and L6 ventral roots but not from the L7 and S1 ventral roots (paper I) while the Ia IPSPs in quadriceps motoneurones

(evoked by Ia afferents in the posterior biceps-semitendinosus nerve) are depressed from the L5 and L6 ventral roots but not from the L - S1 ventral roots This finding gave the first indication that the Ia inhibitory interneurones are recurrently inhibited mainly from efferents to muscles which supply the interneurones with Ia excitation and in a subsequent study in which the effects on Ia IPSPs of stimulation of efferents in several different hindlimb nerves were tested, this was indeed found to be the case (Hultborn et al 1971 b) The pattern of convergence of recurrent inhibition onto Ia inhibitory interneurones and α-motoneurones supplied with the same Ia excitation was so similar that Hultborn et al (1971 b) concluded that the same Renshaw cells seem to project to both groups of neurones

2 To VSCT neurones The VSCT neurones can be divided into three main groups depending on whether they receive monosynaptic excitation from either Ia or Ib afferents or are without monosynaptic excitation from primary afferents (Oscarsson 1957 Eccles et al 1961 a Lundberg and Weight 1971) A fourth smaller group of VSCT neurones seems to receive convergence of monosynaptic excitation from both Ia and Ib afferents (Eccles et al 1961 a, Lundberg and Weight 1971 paper III) Disynaptic Ia IPSPs similar to those in motoneurones are evoked in all these categories of VSCT neurones from nerves to hip and knee muscles most frequently from the nerve to the knee extensor quadriceps (Eccles et al 1961 a, Lundberg and Weight 1971 paper II and III) In the majority of the Ia excited VSCT cells and those without group I excitation the Ia IPSPs were susceptible to recurrent depression from motor axon collaterals (paper II) while the Ia IPSPs in a considerable proportion of the Ib cells and in all the Ia/Ib cells were unaffected (paper III) For the following discussion it is important that disynaptic Ia IPSPs are evoked in Ia VSCT neurones either from the same nerve as the one supplying monosynaptic Ia excitation to the cell or from the nerve to the antagonist muscle of the one supplying the Ia excitation (Lundberg and Weight 1971) In both these combinations the Ia IPSPs were susceptible to recurrent depression (paper II)

The recurrent depression of Ia IPSPs in the VSCT neurones occurred without any recurrent inhibitory effect on the VSCT cells themselves (cf paper IV and section D) The latencies and time courses of the depression were similar to those in motoneurones (cf papers I and II) Further the effect was evoked from α-efferents and IPSPs from the quadriceps nerve were depressed from the L5 and L6 ventral roots but not from the L7 and S1 ventral roots while IPSPs from the posterior biceps-semitendinosus nerve were depressed from the L7 and S1 ventral roots but not from the L5 and L6 ventral roots (papers II and III) There is thus a striking correspondence between the recurrent effects on Ia IPSPs in VSCT neurones and in motoneurones

It was carefully controlled that the negative findings with respect to recurrent effects on Ia IPSPs in some of the Ib VSCT neurones were not due to inadequate testing or to failing preparations In paper III it was therefore concluded that Ia IPSPs are mediated to Ib VSCT neurones by two different groups of Ia inhibitory interneurones with and without recurrent inhibition from motor axon collaterals The two types of Ia IPSPs had similar thresholds and time courses and were evoked from afferents in the same nerve (quadriceps) The only apparent difference between the Ib cells with the two types of Ia IPSPs was in the pattern of Ib excitation In cells with the main Ib excitation from the nerves to hip extensors and knee flexors the Ia IPSPs were recurrently depressed while they were unaffected in all the other Ib VSCT cells At present it is very difficult to interpret the functional significances of these findings (cf Discussion paper III) However one important conclusion to be drawn is that seemingly similar postsynaptic potentials evoked in VSCT neurones from the same kind of afferents in the same nerve may have an entirely different function

3 To DSCT neurones Disynaptic Ia IPSPs are frequently evoked also in DSCT neurones with monosynaptic excitation from group I muscle afferents (Curtis et al, 1958 Eccles et al 1961 b Lindström and Takata 1973) The susceptibility of such IPSPs to recurrent depression from motor axon collaterals has been tested and in no case there was any trace of recurrent effects onto them (Lindström and Takata 1973) The Ia inhibition of the DSCT neurones is thus relayed by other interneurones than the Ia inhibition of motoneurones and most VSCT neurones Although some Ib VSCT cells receive Ia IPSPs which are unaffected by ventral root volleys it is not likely that this effect is mediated by the Ia inhibitory interneurones projecting to the DSCT cells because of the difference in convergence onto the cells The latter often receive convergence of Ia inhibition from several hindlimb nerves and testing with spatial facilitation has revealed that this convergence occurs already at the interneuronal level (Lindström and Takata 1973) The Ia IPSPs in the Ib VSCT neurones on the other hand, are with few exceptions evoked exclusively from the quadriceps nerve The location of the interneurones which mediate the Ia inhibition to the DSCT neurones is unknown It is however possible that these cells are located close to the DSCT neurones in Clarke s column, since interneurones with monosynaptic Ia excitation have been found in this region (Lindström and Takata, unpublished)

4 To segmental interneurones The occurrence of Ia IPSPs in segmental interneurones has not been systematically studied but such effects have been observed in interneurones in the intermediate region of the spinal cord (Ilongo Jankowska and Lundberg 1966 1972) A small number of such interneurones (all with monosynaptic group I

excitation) have been tested with respect to recurrent eff
Ia IPSPs and in all cases these IPSPs were unaffected by
root volleys (Lindström and Schomburg unpublished)

Of special interest for the following discussion is th
ly inhibited Ia interneurones in the ventral horn, which
cal Ia inhibition to motoneurones (Hultborn et al 1971 a
Roberts 1972 b) also receive disynaptic Ia IPSPs The
evoked from nerves to antagonist muscles of those supp
to the interneurones (Hultborn et al 1971 a Hultborn 19
and Schomburg unpublished) These Ia IPSPs are depr
root volleys which indicates that Ia inhibitory interneur
to antagonistic motoneurones mutually inhibit each other

5 To Ia primary afferents Impulses in flex
evoke primary afferent depolarization in Ia afferent terr
Magni and Willis 1962) presumably through a polysyn
The recurrent effects on the Ia interneurones in this pr
conditioning dorsal root potentials evoked by a short tr
in a flexor nerve (paper I) In no case were these dors
affected by a single or a short train of ventral root v
cates that the Ia interneurones in the pathway from Ia
afferent terminals do not receive recurrent inhibit
collaterals

B Selectivity in the recurrent control of Ia int

The question of a selective recurrent control of
neurones was recently discussed by Hultborn (1972
present study has brought some additional informat
by summarized once more The striking finding
experiments (paper I) was that Ia IPSPs evoked in
motoneurones were effectively depressed by volleys
while IPSPs from Ib afferents or different types of
unaffected Occasionally small polysynaptic IPSPs
high threshold muscle afferents and/or cutaneous
depressed but the effects were always less pron
of comparable size

High threshold muscle afferents cutaneous
hold joint afferents frequently evoke similar syn
neurones and are therefore often grouped together
ents (FRA, R M Eccles and Lundberg 1959
1961) The FRA had been found to facilitate the

inhibitory pathway to motoneurones (cf Lundberg 1970) and to activate the recurrently inhibited Ia interneurones in the ventral horn (Hultborn et al. 1971 a). Further the occasional depression of FRA IPSPs in the motoneurones was evoked from the same ventral roots as the depression of the Ia IPSPs in the cells It was therefore suggested in paper I that the recurrently affected FRA IPSPs were partly mediated via the Ia inhibitory interneurones and thereby susceptible to recurrent depression

This suggestion has received strong support from results reported by Fedina and Hultborn (1972 cf also Hultborn 1972 a) They found in different types of preparations a strong positive correlation between the susceptibility of FRA IPSPs to recurrent depression and the ability of FRA volleys to facilitate the transmission in the Ia inhibitory pathway to motoneurones Likewise the susceptibility of IPSPs evoked in motoneurones from different descending systems (cortico- rubro- and vestibulospinal tracts as well as a presumed reticulospinal pathway descending in the medial longitudinal fascicle) to recurrent depression correlates well with the ability of these systems to facilitate transmission in the Ia inhibitory pathway (Hultborn and Udo 1972 b c Hultborn 1972 a) There is thus so far no examples of recurrent effects on IPSPs in motoneurones either from primary afferents or descending tracts which cannot be accounted for by an excitatory convergence from these systems onto the Ia inhibitory interneurones All these findings would indicate that the recurrent effects on segmental reflex pathways to motoneurones are restricted to inhibition of the interneurones in the reciprocal Ia inhibitory pathway as suggested in paper I

There seems to be one exception to this rule Shortly after paper I was submitted for publication there appeared a paper by Ryall (1970) in which he gave evidence for mutual inhibition between Renshaw cells (cf also Ryall and Piercey 1971 Ryall, Piercey and Polosa 1971 1972) Previous scattered observations indicate that Renshaw cells can be fired also by other routes than the well known excitatory path from motor axon collaterals (Eccles et al 1954, Frank and Fuortes 1956 Curtis, Phillis and Watkins 1961, Curtis and Ryall 1966) Accordingly it seems possible that they transmit inhibition from primary afferents which would thus be susceptible to recurrent depression The fact that such a relay through Renshaw cells has not been needed to account for recurrent effects on different types of IPSPs may indicate either that the orthodromic excitation of Renshaw cells is very weak or that the mutual inhibition between them is weak (cf Hultborn 1972 a). Since the present study started from the finding that the recurrent facilitation of motoneurones is a disinhibition, it might be appropriate to mention that Hultborn et al (1971 c) found that this effect largely can be accounted for by the recurrent inhibition of the Ia inhibitory interneurones

Turning to VSCT neurones the results with respect to recurrent effects on IPSPs from other afferent systems than the Ia are entirely in accordance with the findings from motoneurones (papers II and III) Thus Ib IPSPs were unaffected by ventral root volleys while ipsi- and contralateral FRA IPSPs were depressed in some cells It is noteworthy that recurrent depression of FRA IPSPs were found only in VSCT cells with Ia IPSPs susceptible to recurrent depression and not in a large sample of VSCT neurones lacking such inhibition In cells with recurrently affected FRA IPSPs it was possible to facilitate the Ia IPSPs from the FRA, just as was the case in motoneurones Also some disynaptic descending IPSPs were recurrently depressed in cells with Ia IPSPs In unanaesthetized preparations recurrent facilitatory potentials were evoked in VSCT neurones with Ia IPSPs but only from the ventral roots which depressed the Ia IPSPs in the cells Since all these effects were found in cells lacking recurrent inhibition from motor axon collaterals the recurrent effects on interneuronal transmission can also in the case of VSCT neurones be explained by a selective action on the interneurones which mediate Ia inhibition

The observation that dorsal root potentials evoked by Ia volleys were unaffected by ventral root stimulation (paper I; section A, 5) suggested that not all Ia excited interneurones were inhibited from Renshaw cells Confirmatory evidence was obtained from recordings of monosynaptically Ia excited interneurones in the intermediate region which were found to lack recurrent inhibition (Hultborn et al 1971 a) It is not known whether these cells are excitatory or inhibitory However the existence of Ia inhibitory interneurones without recurrent inhibition is proved by the finding that Ia IPSPs in certain Ib VSCT neurones are unaffected by ventral root volleys (paper III; section A ?) This conclusion has been further supported by observations from DSCT neurones (section A, 3) and some group I interneurones in the intermediate region (section A, 4) Thus susceptibility to recurrent inhibition from motor axon collaterals is not a general property of Ia inhibitory interneurones

C Is the Ia inhibition of VSCT neurones and α motoneurones mediated by the same interneurones ?

The study of the recurrent control of the Ia inhibitory pathways to VSCT neurones was initiated in order to test the postulate that the Ia inhibition in these cells is evoked through collateral connexions of the interneurones which relay Ia inhibition to motoneurones (Lundberg 1971) The most direct test would be to record from the interneurones and their

postulated target neurones in order to find if action potentials in the former give rise to unit IPSPs in both motoneurones and VSCT neurones Even if simultaneous recordings from Ia inhibitory interneurones and their target motoneurones have been obtained (Jankowska and Roberts 1972 b) similar recordings from a triplet of neurones are hardly feasable especially when considering that Ia IPSPs are evoked only in some VSCT neurones Any conclusions with respect to the Ia inhibitory relay to VSCT neurones therefore have to rest on indirect evidence as obtained in the present study

Clearly the finding that the Ia IPSPs in many VSCT neurones are susceptible to recurrent depression from motor axon collaterals is in full accordance with the idea of a common interneuronal origin of the Ia IPSPs in VSCT neurones and motoneurones The other possible explana tion for the findings namely two parallel Ia inhibitory pathways one directed to the VSCT neurones and the other to motoneurones and both being recurrently controlled, is extremely unlikely (cf paper II) Also from a functional point of view the idea of a common interneurone seems much more attractive Even if the function of the recurrent control of the Ia inhibitory pathway to motoneurones is not fully understood this action presumably plays a role in the regulation of the depth of the reciprocal inhibition of motoneurones (Hultborn et al 1972 b Hultborn and Lundberg 1972 Hultborn 197_ a b) The mere fact that the VSCT receives Ia inhibition which is regulated by the motor output in the same way as Ia inhibition to motoneurones strongly suggests that its information transfer is related to this inhibitory pathway The most simple coupling would clearly be collateral connexions from the interneurones in the Ia inhibitory pathway to motoneurones

D Recurrent inhibition of VSCT neurones from motor axon collaterals

Since the findings with respect to the Ia inhibitory pathway to VSCT neurones were consistent with the idea that the VSCT conveys information about this pathway it was deemed important (cf Discussion) to complete the picture by searching for recurrent inhibition of the VSCT neurones from motor axon collaterals In paper IV was reported that IPSPs are indeed evoked in some VSCT neurones on antidromic stimulation of ventral roots The IPSPs increased in amplitude with the α-volley had segmental latencies indicating a disynaptic linkage from the motor axons to the VSCT neurones and time courses similar to those of recurrent IPSPs in motoneurones and Ia inhibitory interneurones (cf *Eccles et al* 1954 Hultborn et al 1971 a) It is thus likely that the pathway is similarly organized as the recurrent inhibitory path to the latter cells

It is notable that all the recurrently inhibited VSCT neurones received monosynaptic excitation from Ia afferents and that in the Q excited cells the recurrent inhibition was evoked from the L5 and L6 ventral roots but not from the L7 and S1 ventral roots just as is the case for Q excited α-motoneurones and Ia inhibitory interneurones (paper I and Hultborn et al 19 1 a) Recurrent inhibition was found only in a very small fraction of the recorded Ia VSCT neurones but it should by no means be assumed that this connexion is aberrant (cf Discussion paper IV) Rather the small size of the pool of recurrently inhibited VSCT neurones is taken to indicate a high degree of selectivity in connexions to individual VSCT neurones

For the interpretation of the functional significance of the recurrent inhibition to VSCT neurones it is essential to learn whether it is mediated by the same interneurones which transmit recurrent inhibition to motoneurones and Ia inhibitory interneurones It has earlier been mentioned that there is good reason to assume that the same Renshaw cells project to α-motoneurones and Ia inhibitory interneurones excited from Ia afferents in the same nerve On the basis of the correspondence in the convergence onto the VSCT neurones and of similar functional considerations as in previous section it seems reasonable to postulate that the same Renshaw cells also project to VSCT neurones

E Composition of the VSCT neuronal pool with respect to monosynaptic excitation from muscle afferents

The first studies on the VSCT led to the conclusion that the only primary afferents supplying monosynaptic excitation to VSCT neurones were the Golgi tendon organ (Ib) afferents (cf Oscarsson 1965 b) This conclusion was based on mass discharge recordings from the tract (Oscarsson 1956 1957) intraaxonal recordings (Oscarsson 1957 Lundberg and Oscarsson 1962) and intracellular recordings from the cell bodies (Eccles et al 1961 a). Of the VSCT neurones found in the latter two types of experiments a considerable proportion did not receive monosynaptic excitation from any of the dissected muscle or cutaneous nerves The vast majority however received strong polysynaptic effects predominantly inhibition, from the FRA The cell bodies of the Ib VSCT neurones were found scattered in a region dorsomedially to the motor nuclei (Hubbard and Oscarsson 1962)

Later it has been found that a group of cells along the lateral border of the ventral horn, apparently identical with the spinal border cells of Cooper and Sherrington (1940) also belong to the VSCT (Burke et al 1971) A number of these cells are monosynaptically

excited from large muscle spindle (Ia) afferents while Ib excited cells are only occasionally encountered in this region (Lundberg and Weight 1971) Many of the spinal border cells are also without monosynaptic excitation from the commonly dissected hindlimb nerves (cf Lundberg and Weight 1971 and paper II) and in experiments with stimulation of the dorsal columns it was proved that practically all the latter cells entirely lacked monosynaptic excitation from primary afferents (Lundberg and Weight 1971)

The sampling of different types of VSCT neurones in the studies of Eccles et al (1961 a) and Lundberg and Weight (1971) probably was due to the use of different microelectrode approaches in the two studies The insertion of the microelectrodes from the dorsal column (Eccles et al,(1961 a) favoured the exploration of the region dorsomedially to the motor nuclei where the Ib cells are common, while the insertion of the microelectrodes laterally to the dorsal root entry zone (Burke et al 1971 Lundberg and Weight 1971) made the spinal border cell region more accessible

In the present study (paper II) the microelectrodes were usually inserted from the dorsal column but with the knowledge of the more laterally located VSCT neurones they were angled to cover both the medial and lateral areas containing VSCT neurones It is thus likely that the sampling of neurones in this case was more representative than in the earlier studies Of more than 140 intracellularly recorded VSCT neurones about 25 per cent received mainly Ia excitation, 15 per cent mainly Ib excitation 10 per cent group I excitation which could not be classified as evoked from either Ia or Ib afferents while about 45 per cent were without monosynaptic excitation from any of the dissected nerves In a few additional per cent of cells there seemed to be convergence of monosynaptic excitation both from Ia and Ib afferents

If anything the figure for cells without monosynaptic excitation from group I afferents is underestimated since these cells seem to be relatively more common in the L3 and L4 segments (Burke et al 1971) which were not so extensively explored in this study The figure for Ib VSCT neurones would not be much underestimated since in the experiments especially devoted to record from Ib VSCT neurones (paper III) less than 40 per cent of the impaled cells received monosynaptic Ib excitation (Lindström and Schomburg unpublished) Provided no large group of VSCT neurones remains to be detected the results thus indicate that roughly a fourth of the VSCT neurones are monosynaptically excited from Ia afferents another fourth monosynaptically excited from Ib afferents, while the remaining cells lack monosynaptic excitation from primary afferents

These results should be correlated with anatomical findings of retrograde degeneration after spinal cord transections The majority of cells showing retrograde changes after such operations are found along the lateral border of the ventral horn where the Ia VSCT neurones and those lacking group I excitation mainly are located, while only scattered cells showing chromatolysis are found in the more dorsal region where the Ib VSCT neurones are more common (Cooper and Sherrington 1940 Sprague 1953) With the reservation that not all cells with transected axons may show chromatolysis with the usual techniques, these findings seem to be in general agreement with the electrophysiological results

When looking at the detailed pattern of effects to the VSCT it is apparent that each of the above groups consists of several subgroups For instance, there are Ia VSCT cells which receive only Ia excitation or Ia excitation and inhibition from the same nerve, or reciprocally organized Ia excitation and inhibition or Ia excitation with recurrent inhibition from motor axon collaterals, or Ia excitation combined with Ib excitation (Lundberg and Weight 1971 papers II–IV) Each such subgroup may constitute a very small fraction of the total VSCT pool The diversity would be even greater if effects from other afferent systems and descending tracts also were considered This clearly indicates that each individual VSCT cell carries a very specific piece of information It is also understandable that this highly differentiated input led Lundberg and Weight (1971) to conclude that one cell hardly resembles another

GENERAL DISCUSSION

A Comments on the functional significance of the recurrent control of the Ia inhibitory pathway to motoneurones

The present results indicate that the recurrent control of reflex transmission from primary afferents to motoneurones is restricted to the interneurones in the inhibitory pathway from large muscle spindle (Ia) afferents to motoneurones This conclusion has found strong support in subsequent studies in which also the recurrent control of descending effects was analyzed (cf Hultborn 1972 a) Since the recurrent inhibition of α-motoneurones is distributed primarily between motor nuclei coupled in Ia synergism (Eccles et al 1961 c Hultborn et al 1971 b) and since motoneurones and Ia inhibitory interneurones supplied with the same Ia excitation receive a similarly organized recurrent inhibition (Hultborn et al, 1971 b) it seems likely that the recurrent inhibition from motor axon collaterals is related to the γ-control of movements This idea is supported by the recent finding that also some γ-motoneurones receive recurrent inhibition from motor axon collaterals (Ellaway 1968 Brown, Lawrence and Matthews 1968 Grillner 1969 Ellaway 1971 Noth 1971)

The idea that many movements depend on a coactivation of α- and γ motoneurones developed from the finding that several neuronal path ways evoke parallel effect in α and γ motoneurones (Granit 1955 1970 Grillner 1969 Matthews 1972) Such a coactivation is found in a number of natural movements: breathing (Eklund, Euler and Rutkowski 1964, Sears 1964) stepping (Severin, Orlovsky and Shik 1967) jaw movements (Taylor and Davey 1968) and voluntary finger movements in man (Hagbarth and Vallbo 1968 1969 Vallbo 1971) In all these cases the balance between the α- and γ-activation is set so that the afferent discharge from the muscle spindles actually increases during muscle contraction These findings indicate that the activation of motoneurones may depend on spatial facilitation between a more direct α-route of excitation and an indirect γ-route via the muscle spindles In these movements the increase in spindle afferent discharges often begins after the onset of the α-activity and regular respiratory and walking cycles can occur also after deafferentation although the muscle strength is impaired Accordingly these movements rather than being generated through a "follow-up length" servo operated by the γ-system (Merton 1953) can be considered to depend on α-γ-linkage and receive "servo assistance" from the γ system (cf Matthews 1972)

It is likely that reciprocal inhibition from Ia afferents plays a role in many types of movements It has been pointed out that neuronal systems which supply parallel excitation to α- and γ-motoneurones also excite the interneurones in the Ia inhibitory pathway (Hongo et al 1969 Lundberg 1970) Thus the activity in the Ia inhibitory interneurones seems to depend on spatial facilitation between excitation through a more direct route, corresponding to the α-route to motoneurones and through the indirect γ-route via Ia afferents Those authors pointed out the advantage in certain situations of a parallel regulation of the excitation of α-motoneurones and of the interneurones relaying reciprocal inhibition to antagonist motoneurones In order to stress the similarity in the regulation of the activity in motoneurones and Ia inhibitory interneurones they coined the term α-γ-linked reciprocal inhibition

The need for reciprocal inhibition may vary in different types of movements and implicit in the idea of α-γ-linked reciprocal inhibition lies the possibility for control of reciprocal inhibition by other neuronal systems than Ia afferents The interneurone interposed in the Ia inhibitory pathway may thus serve as an integrative station

In order to understand the functional significance of Ia reciprocal inhibition it is necessary to consider the distribution of Ia actions The "myotatic unit" in the terminology of Lloyd (1946 b) was originally believed to include only muscles acting as synergists and antagonists at the same joint R M Eccles and Lundberg (1958 b) however found a wider distribution of Ia actions from some muscles and suggested that this extended Ia pattern has developed to assist locomotion (cf also Engberg and Lundberg 1969 Lundberg 1969 b) In this connexion it is of interest that reciprocal Ia inhibition seems to operate only between muscles acting as flexors and extensors of different joints but not between abductors and adductors (R M Eccles and Lundberg 1958 b Hongo et al 1969) The role of the reciprocal inhibition in the alternating activation of flexors and extensors in locomotion might be to eliminate stray excitatory effects during the passive phase of the step cycle and to cut off outlasting Ia excitation during the shifts between flexion and extension phases

On the other hand, the wide distribution of excitatory and inhibitory Ia actions may rather be a disadvantage during more precise movements engaging only a single muscle or requiring cocontraction of muscles operating as antagonists at the same joint Since the recurrent inhibition to motoneurones and Ia inhibitory interneurones "cover" this extended Ia pattern Hultborn et al (1971 b) suggested that the role of the recurrent inhibition may be to limit the diverse Ia effects thereby subserving the γ-control of precise movements or postures, perhaps involving cocontraction of antagonist muscles This idea presupposes that the Renshaw cells

can be controlled from other segmental and descending sources than the motor axon collaterals (for reference see paper IV) which provides a possibility to regulate the amount of recurrent inhibition produced by a given motor output

On the basis of the above consideration regarding the role of reciprocal Ia inhibition in stepping it is interesting that there is evidence for less effective transmission in the recurrent inhibitory pathway during stepping In mesencephalic cats which perform locomotion on a treadmill during stimulation of the "locomotor region" (Shik Orlovsky and Severin 1966) the recurrent inhibitory pathway to motoneurones is depressed during locomotion (Severin Orlovsky and Shik 1968) In this connexion it also deserves to be mentioned that a similar depression can be evoked after DOPA (Bergmans Burke and Lundberg 1969) This drug liberates transmittors from a descending noradrenergic pathway and thereby releases an interneuronal network with mutual inhibitory connexions between interneuronal pools supplying excitation to extensors and flexors respectively (Jankowska, Jukes Lund and Lundberg 1967) It has been suggested that this interneuronal network serves as the segmental generator for the alternating activation of flexors and extensors in locomotion It has recently been found (Fu, Jankowska and Lundberg unpublished observation) that FRA volleys which can activate either of these interneuronal pools also evoke excitatory action in the Ia inhibitory interneurones projecting to antagonist motoneurones (this pathway is not shown in Fig 1) This finding provides another example of α-γ-linked reciprocal inhibition After DOPA FRA volleys may evoke reciprocal inhibition and it is worth noting that these reciprocal IPSPs are almost entirely removed by volleys in the appropriate ventral roots suggesting that the inhibition is mediated entirely through the Ia inhibitory interneurones Thus associated with the activation of the spinal centres generating alternating activation of flexors and extensors there seems to be a regulation both of the pathway mediating reciprocal inhibition of motoneurones and of the recurrent inhibitory pathway controlling this reciprocal inhibitory pathway

The parallel recurrent control to α-motoneurones and Ia inhibitory interneurones (presumably through the same Renshaw cells Hultborn et al 1971 b) should not be taken to indicate that the resulting effects necessarily are equivalent It seems likely that the degree of reciprocal inhibition largely depends on regulation of the firing frequency of the Ia inhibitory interneurones while the regulation of the amount of motor activity largely depends on recruitment of new motor units (Severin, Shik and Orlovsky 1967 Grillner and Udo 1971) In case of motoneurones the recurrent inhibition evoked by the first mobilized cells will tend to increase the amount of excitation required to activate other motoneurones The recurrent inhibition of the Ia inhibitory interneurones, on the other hand, may function by adjusting the average firing frequency

of the interneurones to a level appropriate for the intended movement It is obvious that the suggested regulatory effect of the recurrent inhibition in relation to the reciprocal inhibition should not be seen as an all or nothing phenomenon Even in cocontraction an appropriate balanced reciprocal inhibition might be desirable For further aspects on the functional significance of the recurrent inhibition of Ia inhibitory interneurones see Hultborn and Lundberg (1972) and Hultborn (1972 a, b)

B Information transfer in the VSCT

The existence of two direct spinocerebellar pathways from the hindlimb region has been known since the end of the last century although there have been controversies with respect to the location of the cell bodies and the spinal course of the tracts (cf Smith 1957 Jansen and Brodal 1958) It is now well established that the dorsal spinocerebellar tract (DSCT) contains neurones which are monosynaptically excited from afferents of muscle spindles Golgi tendon organs joint receptors and different types of cutaneous receptors (Oscarsson 1965 b Lindström and Takata 1972) some of them receive convergence of excitation from different types of receptors while others seem to obtain a modality specific input The excitation of the DSCT cells is often supplied from restricted receptive fields for instance a single muscle or a part of a toe pad Although a still poorly understood integration seems to occur at the DSCT cell level, this tract can be assumed to convey specific information from peripheral receptors to cerebellum

The initial physiological studies on the ventral spinocerebellar tract (VSCT) by Oscarsson (1956 1957) were concerned with neurones monosynaptically excited from Golgi tendon organ (Ib) afferents These cells also received strong polysynaptic effects from the FRA and the FRA effects could be markedly influenced from several descending systems (Oscarsson 1965 b) Surprisingly and contrasting with the strong excitatory coupling to DSCT neurones the Ib VSCT neurones were very weakly driven by adequate activation of Golgi tendon organs of a single muscle (Oscarsson 1960) It was rather difficult to account for these findings and Oscarsson (1957 1965 b) suggested in general terms that the VSCT neurones might forward information concerning movement or posture of a whole limb and that the descending control of the pathways from the FRA to VSCT neurones might allow selection of information from either tendon organ afferents or flexor reflex afferents

With the finding that the spinal border cells of Cooper and Sherrington (1940) belong to the VSCT (Burke et al 1971) it became clear that the VSCT consists of a nonhomogeneous population of neurones

some being monosynaptically excited from Ia afferents others from Ib afferents and some entirely lacking monosynaptic excitation from primary afferents (Lundberg and Weight 1971) The VSCT neurones also receive mono- and polysynaptic excitation and inhibition from several descending tracts (Oscarsson 1965 b Baldissera and Bruggencate 1969 Baldissera and Weight 1969) Lundberg (1971) noted a similarity in the convergence of excitation and inhibition from different types of primary afferents and descending tracts on segmental interneurones and on the VSCT neurones and forwarded the hypothesis that the VSCT relays information about the transmission in interneuronal reflex pathways to motoneurones This hypothesis should be considered in the perspective of the extensive convergence from different segmental and descending systems on the interneurones in these reflex pathways as exemplified in Fig 1 for the reciprocal Ia inhibitory pathway to motoneurones (cf Hultborn 1972 a b) It seems reasonable to assume that the descending control of motoneurones which is exerted mainly through such interneurones (Lundberg 1966 1969 a Hongo et al 1972 Hultborn 1972 a, b) would require feedback information regarding the transmission in these pathways

The idea that ascending pathways may relay information about interneuronal transmission to motoneurones is not new The possibility has been discussed earlier in relation to effects from the FRA on ascending pathways (Lundberg 1959 1964 Oscarsson 1967 1968 Miller and Oscarsson 1970) among them the VSCT which receives strong FRA effects (Oscarsson 1965 b Lundberg and Weight 1971) Lundberg s new hypothesis is, however very attractive for the following reasons Firstly it offers a plausible explanation for the existence of two direct spinocerebellar pathways from the hindlimb region, the DSCT signalling information about the periphery and the VSCT about the activity in the spinal cord at the segmental level Secondly the hypothesis comprises one of the simplest and best known interneuronal reflex pathways in the spinal cord the disynaptic Ia inhibitory pathway to motoneurones and is therefore accessible to experimental testing Thirdly the hypothesis gives a detailed account of possible mechanisms for the information transfer through the VSCT about interneuronal reflex pathways

A central idea in the hypothesis is that VSCT neurones receive information about the activity in the interneuronal chains through collateral connexions, both from the last order interneurones in the pathways and from excitatory and inhibitory neurones impinging onto these interneurones With respect to the Ia inhibitory pathway this idea has found strong support in the present study It seems therefore appropriate to consider in some detail how the VSCT may convey information about interneuronal reflex pathways using the Ia inhibitory pathway as a model

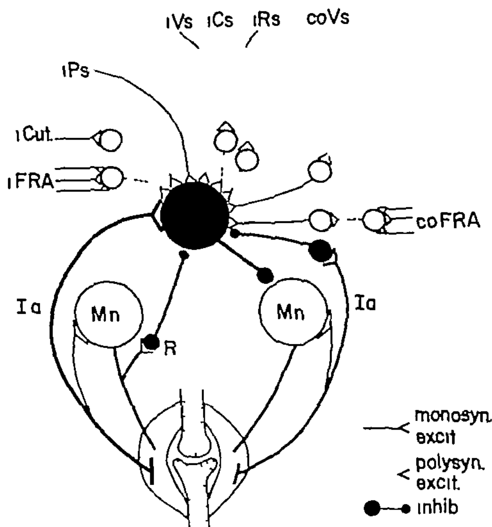


Fig 1 Schematic representation of some synaptic connexions to the interneurones in the inhibitory pathway from large muscle spindle (Ia) afferents to α-motoneurones (cf Hultborn 1972 a, b) i, ipsilateral; co contralateral; Vs Cs, Rs Ps, vestibulo- cortico rubro- and propriospinal tracts respectively; Ia, Ia afferents; Cut cutaneous afferents; FRA, flexor reflex afferents Mn motoneurones; R, Renshaw cells; ant, antagonist

For the descending regulation of a given Ia inhibitory pathway to motoneurones it should be of crucial importance to know the exact amount of inhibition which the interneurones in this pathway exert on their target motoneurones This information could be obtained by collateral connexions to VSCT neurones from the Ia inhibitory interneurones However since the output of these interneurones is in the form of inhibition, and the information has to be conveyed as spike activity the inhibition has to be displayed against some background activity in the VSCT neurones Such background activity could be generated by a pacemaker mechanism in the VSCT neurones or by some constant unspecific excitatory input to them but there are no indications of such arrangements for the VSCT The problem seems to have been solved in the way that the VSCT neurones are supplied with the same Ia excitatory input as the Ia inhibitory interneurones again through collateral connexions (Fig 2 A) This is an input-output comparator cell for the Ia inhibitory pathway (Lundberg and Weight 1970 Lundberg 1971) and in reality this type of cell is represented by the VSCT neurones with convergence of monosynaptic excitation and disynaptic inhibition from Ia afferents in the same nerve (cf paper II)

Such an input-output comparator cell might give an adequate information about the activity of the Ia inhibitory interneurones provided all excitatory and inhibitory neurones terminating on them also send collaterals to the VSCT cell This seems however not to be the case, presumably because this information would be unsufficient for cerebellum On the other hand, the information content in the firing of a single input-output comparator cell is considerably reduced if some of the inputs to the Ia inhibitory interneurones are missing to the VSCT cell This is illustrated schematically in Fig 2 B Assume that only the Ia excitatory input is fed to the VSCT neurone together with the output from the interneurones A certain relationship may then be found between the Ia excitation and the firing frequency of the input output comparator cell as indicated by curve a If the Ia inhibitory interneurones in addition are inhibited from some source, then the excitation of the VSCT neurone will be less effectively counteracted by the firing of the Ia inhibitory interneurones The relation between the Ia input and the firing frequency of the VSCT cell will then change as shown by curve b If the Ia inhibitory interneurones instead receive excitation from some source other than the Ia afferents they will fire more intensely for a given Ia input and give the relation depicted by curve c Under such conditions it would be impossible to determine from the firing frequency of the input-output comparator cell if the Ia inhibitory interneurones receive a weak Ia excitation combined with inhibition (resulting in negligible Ia inhibition of the motoneurones) or a strong Ia excitation with additional excitatory impingements (giving profound Ia inhibition of the motoneurones, cf dotted line in Fig 2 B)

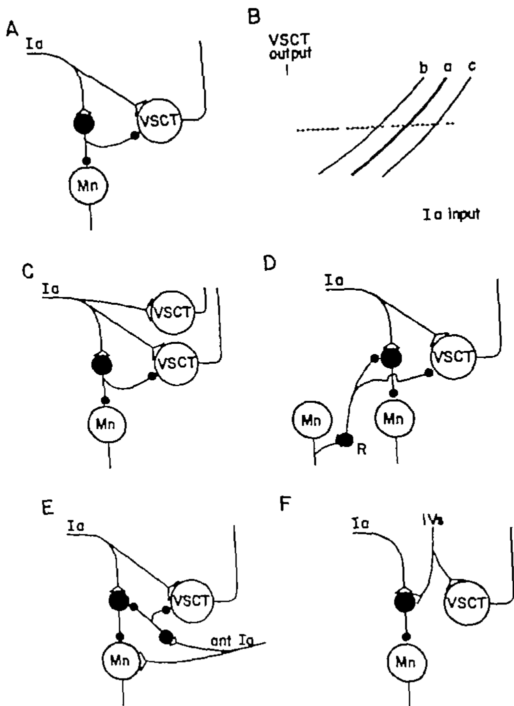


Fig 2 Schematic representation of different connexions to ventral spinocerebellar tract neurones See text.

Such a difficulty might be overcome by having in parallel other input-output comparator cells which receive the same effects but in different quantities A more interesting alternative, which is in keep ing with experimental observations, is depicted in Fig C Parallel with the input-output comparator cell there is another VSCT neurone which receives only Ia excitation corresponding to that of the Ia in hibitory interneurones By subtracting the firing of the input-output comparator cell from that of the other VSCT cell cerebellum would obtain a measure of the inhibitory output from the Ia inhibitory interneurones At the same time information about the Ia excitation supplied to these interneurones would be available

Theoretically the Ia excitation and inhibition of the input output comparator cells could be balanced so that the discharge frequency of these cells remained at a constant level independently of changes in the Ia excitation of the Ia interneurones (that is curve a in Fig 2 B could be horizontal) Any extra excitation of the Ia inhibitory interneurones from sources other than the Ia afferents would then be measured as a decrease in the firing frequency of the input-output comparator cells and inhibition of the interneurones would be measured as an in crease in the frequency However since the Ia inhibition of the motoneurones varies with the Ia excitation of the interneurones it would also in such cases be essential with an extra line to cerebellum for the Ia input, as shown in Fig 2 C

For a proper regulation of the Ia inhibitory pathway cerebellum probably needs a detailed information also about the contribution of excitation and inhibition from other neuronal systems to the Ia inhibitory interneurones (cf Fig 1) Recurrent inhibition from motor axon collaterals and inhibition from other Ia inhibitory interneurones are two examples of such inputs to the Ia inhibitory interneurones The present results indicate that information also about these effects may be available for cerebellum through the VSCT Some VSCT neurones receive recurrent inhibition from motor axon collaterals and this effect is in all likelihood mediated through collaterals of Renshaw cells which project to the Ia inhibitory interneurones Again the information is in the form of inhibition and also *in this case the inhibition seems to* be displayed against Ia excitation corresponding to that of the Ia in hibitory interneurones (Fig 2 D; cf paper IV) As in the case of the previously discussed input-output comparator cell, cerebellum could extract information about the amount of recurrent inhibition to the Ia inhibitory interneurones by comparing the output of this VSCT cell with that of a cell excited exclusively from Ia afferents In a similar way information about the mutual Ia inhibition of the Ia inhibitory interneurones seems to be conveyed by VSCT neurones with reciprocally

organized Ia excitation and inhibition (Fig 2 E; of paper II) The results with respect to other segmental inhibitory inputs to the Ia inhibitory interneurones are in principle compatible with the suggested scheme, but the organization of these inputs to the Ia inhibitory interneurones is not sufficiently well known to allow us to push the comparison any further (cf paper II)

Information about excitatory inputs to the Ia inhibitory interneurones is probably signalled in an analogous way There may be, however one important difference Since excitatory neurones through their collaterals will supply the VSCT cells with excitation, there would be no need to combine this input to the VSCT cells with Ia excitation This is exemplified in Fig 2 F with effects from the vestibulospinal tract which has been found to evoke monosynaptic excitation in some VSCT cells lacking effects from group I muscle afferents (Baldissera and Roberts unpublished observation) However the vestibulospinal tract (unfortunately!) has monosynaptic connexions also with other segmental interneurones (Bruggencate, Burke Lundberg and Udo 1969) It is therefore impossible to tell whether VSCT cells like the one in Fig 2 F really are concerned with the reciprocal Ia inhibitory pathway since they lack the marking with Ia excitation The same difficulty would apply to other excitatory inputs to the Ia inhibitory interneurones On the other hand, VSCT neurones with Ia IPSPs susceptible to recurrent depression but without monosynaptic excitation from group I afferents (paper II) are likely to be related with some of the descending excitatory inputs to the Ia inhibitory interneurones

Certainly the above discussion gives a simplified description of the mechanisms for the transfer of information about the reciprocal Ia inhibitory pathway to motoneurones through the VSCT For instance, cells can be found which in different combinations receive convergent effects corresponding to several of the inputs to the Ia inhibitory interneurones (cf Fig 2 paper IV) However the pattern of convergence on VSCT neurones found in the present study shows that the VSCT has the capability to convey a detailed information about the different inputs to and the output from the interneurones in the reciprocal Ia inhibitory pathway to motoneurones thereby giving a complete account of the inhibitory control of motoneurones through this pathway The results also indicate that this information is relayed fractionated through a set of VSCT neurones each one carrying a part of the total information

Lundberg (1971) discussed at some length how the VSCT may convey information about other inhibitory reflex pathways to motoneurones for instance from Ib afferents or the FRA More direct experimental evidence for an information transfer through the VSCT about these other pathways is still scanty mainly because the organization of these pathways is not known in sufficient detail to allow appropriate testing However

Baldissera and Roberts (unpublished observation) have obtained results indicating that some VSCT neurones are concerned with the last order inhibitory interneurones in the inhibitory pathway to flexor motoneurones from the contralateral FRA which also receive monosynaptic excitation from the vestibulospinal tract Paper III described some observations indicating that VSCT neurones may receive convergence of monosynaptic excitation and disynaptic inhibition from Ib afferents in the same nerve, giving Ib input-output comparator cells On the assumption that Ib excited VSCT neurones may be concerned with Ib pathways to motoneurones and from the observations that these VSCT neurones frequently receive Ia inhibition (Eccles et al 1961 a paper III) it was also suggested that the interneurones in the Ib pathways may be under inhibitory control from Ia inhibitory interneurones If this prediction should prove to be correct it would provide further evidence in support for the discussed VSCT hypothesis It is noteworthy that Ia and Ib afferents, which are the only primary afferents known with certainty to have disynaptic connexions with motoneurones likewise are the only afferents found to connect monosynaptically with the VSCT

Although Lundberg (1971) mainly discussed the VSCT function in relation to inhibitory reflex pathways it should not be excluded that some VSCT neurones may convey information about excitatory interneuronal pathways for instance from Ib afferents or from the FRA In such cases an input-output comparator arrangement would not be required since the excitatory interneurones in these pathways can activate directly the VSCT neurones through collateral connexions, as discussed above for excitatory neurones impinging on the Ia inhibitory interneurones As in that case it might be very difficult to obtain experimental evidence for this possible VSCT function Information about excitatory pathways to motoneurones would be obtained also if the convergence on some VSCT neurones was related to the convergence on motoneurones instead of interneurones (Lundberg 1971) It should be mentioned that since recurrent inhibition and reciprocally organized Ia excitation and inhibition are evoked both in Ia inhibitory interneurones and motoneurones the VSCT cells with the convergence shown in Fig 2 D and E may as well signal information about excitatory and inhibitory actions on motoneurones

In papers II and III the claim of Arshavsky Berkinblit, Gelfand, Orlovsky and Fukson (1972 a) that the rhythmic modulation of the dis charges in VSCT neurones in walking cats remains after deafferentation was considered as a support for the hypothesis that the VSCT is con cerned mainly with interneuronal activity at the segmental level Unfortunately the criteria used by these authors for identification of the VSCT neurones, as described in the full papers (Arshavsky et al.1972 b c) leave some doubt whether the investigated cells really belong to the VSCT It seems therefore necessary to repeat these very interesting experiments

with properly identified units It would certainly also be interesting to know whether the present hypothesis about the function of the VSCT is applicable on the forelimb equivalent the rostral spinocerebellar pathway (Oscarsson and Uddenberg 1964 1965 Oscarsson 1965 a)

At present very little can be said about the cerebellar processing of the information received through the VSCT The finding that each VSCT axon can be antidromically activated at low threshold from a relatively large area of the cerebellar cortex (compared to the DSCT cf Lundberg and Oscarsson 1960 1962 Burke et al 1971) suggesting an extensive preterminal branching of the VSCT mossy fibres may be of relevance in relation to the suggested organization of the VSCT with sets of neurones relying fractionated information about a given reflex pathway The bilateral termination in the cerebellar cortex of some VSCT neurones (Lundberg and Oscarsson 1962 Burke et al 1971) may be correlated with the bilateral termination in the spinal cord of some segmental interneurones Needless to say the information about central activities at the *segmental level received by cerebellum through the VSCT* should not be seen in isolation from information supplied through other channels for instance from the periphery through the DSCT However the intricate organization of the cerebellar cortex with a vast divergence and convergence in the mossy fibre-Purkinje cell loop (Palkovits Magyar and Szentágothai 1972) implies that the cerebellum is capable of decoding" a complicated information submitted through a multiplicity of individual input neurones

Baldissera and Roberts (unpublished observation) have obtained results indicating that some VSCT neurones are concerned with the last order inhibitory interneurones in the inhibitory pathway to flexor motoneurones from the contralateral FRA, which also receive monosynaptic excitation from the vestibulospinal tract Paper III described some observations indicating that VSCT neurones may receive convergence of monosynaptic excitation and disynaptic inhibition from Ib afferents in the same nerve, giving Ib input–output comparator cells On the assumption that Ib excited VSCT neurones may be concerned with Ib pathways to motoneurones and from the observations that these VSCT neurones frequently receive Ia inhibition (Eccles et al 1961 a paper III) it was also suggested that the interneurones in the Ib pathways may be under inhibitory control from Ia inhibitory interneurones If this prediction should prove to be correct it would provide further evidence in support for the discussed VSCT hypothesis It is noteworthy that Ia and Ib afferents which are the only primary afferents known with certainty to have disynaptic connexions with motoneurones likewise are the only afferents found to connect monosynaptically with the VSCT

Although Lundberg (1971) mainly discussed the VSCT function in relation to inhibitory reflex pathways it should not be excluded that some VSCT neurones may convey information about excitatory interneuronal pathways for instance from Ib afferents or from the FRA In such cases an input–output comparator arrangement would not be required since the excitatory interneurones in these pathways can activate directly the VSCT neurones through collateral connexions, as discussed above for excitatory neurones impinging on the Ia inhibitory interneurones As in that case it might be very difficult to obtain experimental evidence for this possible VSCT function Information about excitatory pathways to motoneurones would be obtained also if the convergence on some VSCT neurones was related to the convergence on motoneurones instead of interneurones (Lundberg 1971) It should be mentioned that since recurrent inhibition and reciprocally organized Ia excitation and inhibition are evoked both in Ia inhibitory interneurones and motoneurones the VSCT cells with the convergence shown in Fig . D and E may as well signal information about excitatory and inhibitory actions on motoneurones

In papers II and III the claim of Arshavsky Berkinblit, Gelfand, Orlovsky and Fukson (1972 a) that the rhythmic modulation of the dis charges in VSCT neurones in walking cats remains after deafferentation was considered as a support for the hypothesis that the VSCT is con cerned mainly with interneuronal activity at the segmental level Unfortunately the criteria used by these authors for identification of the VSCT neurones as described in the full papers (Arshavsky et al 1972 b c) leave some doubt whether the investigated cells really belong to the VSCT It seems therefore necessary to repeat these very interesting experiments

with properly identified units It would certainly also be interesting to know whether the present hypothesis about the function of the VSCT is applicable on the forelimb equivalent the rostral spinocerebellar pathway (Oscarsson and Uddenberg 1964 1965 Oscarsson 1965 a)

At present very little can be said about the cerebellar processing of the information received through the VSCT The finding that each VSCT axon can be antidromically activated at low threshold from a relatively large area of the cerebellar cortex (compared to the DSCT; cf Lundberg and Oscarsson 1960 1962 Burke et al 1971) suggesting an extensive preterminal branching of the VSCT mossy fibres may be of relevance in relation to the suggested organization of the VSCT with sets of neurones relying fractionated information about a given reflex pathway The bilateral termination in the cerebellar cortex of some VSCT neurones (Lundberg and Oscarsson 196* Burke et al 1971) may be correlated with the bilateral termination in the spinal cord of some segmental interneurones Needless to say the information about central activities at the segmental level received by cerebellum through the VSCT should not be seen in isolation from information supplied through other channels for instance from the periphery through the DSCT However the intricate organization of the cerebellar cortex with a vast divergence and convergence in the mossy fibre–Purkinje cell loop (Palkovits Magyar and Szentágothai 1972) implies that the cerebellum is capable of "decoding a complicated information submitted through a multiplicity of individual input neurones

SUMMARY AND CONCLUSIONS

1 The effects of impulses in recurrent motor axon collaterals on transmission in inhibitory reflex pathways from different types of primary afferents to α-motoneurones and ventral spinocerebellar tract neurones have been investigated in the cat by conditioning IPSPs evoked in these neurones

2 The transmission in the disynaptic inhibitory pathway from large muscle spindle (Ia) afferents to α-motoneurones of antagonist muscles is inhibited by antidromic impulses in motor axons The effect is due to postsynaptic inhibition of the interneurones interposed in this pathway and is mediated through recurrent motor axon collaterals and Renshaw cells The results indicate that besides Renshaw cells themselves these interneurones are the only segmental interneurones influenced from motor axon collaterals through Renshaw cells

3 The transmission in the disynaptic Ia inhibitory pathways to some (but not all) ventral spinocerebellar tract neurones is also inhibited at the interneuronal level by antidromic impulses in motor axon collaterals It is concluded that the Ia inhibition of these cells and of α-motoneurones is mediated by the same interneurones

4 Disynaptic recurrent inhibition from motor axon collaterals is evoked in some ventral spinocerebellar tract neurones monosynaptically excited from Ia afferents The effect is presumably mediated through collateral connexions from the same Renshaw cells which inhibit α-motoneurones and Ia inhibitory interneurones

5 The results with respect to the ventral spinocerebellar tract strongly indicate that this tract is capable of signalling information about the transmission in segmental reflex pathways to α-motoneurones Possible mechanisms for this information transfer are discussed, using the Ia inhibitory pathway as a model

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to my teacher Anders Lundberg for many inspiring discussions and for invaluble help and support throughout this investigation

I also wish to thank Bengt Gustafsson Hans Hultborn, Elzbieta Jankowska and Elke Schomburg for a very stimulating collaboration I owe special thanks to Elzbieta Jankowska for comments on the different manuscripts

Thanks are also due to Erling Eide with collaborators and Yngve Källström for their excellent technical constructions to Rami Larsson for a particularly competent technical assistance to Tomas Palm for preparation of the illustrations and to Berit Nilsson for skilful typing of the manuscripts

Lastly I wish to thank all members of the staff for creating a friendly and stimulating atmosphere in the department.

This investigation was supported by grants from the Swedish Medical Research Council (Projects No B70 14X 94-06B and B73 14X-94-09B) the Medical Faculty University of Göteborg Svenska Sällskapet för Medicinsk Forskning and Magnus Bergvalls Stiftelse

ANDEN N -E M G M JUKES A LUNDBERG and L VYKLICKY The effect of DOPA on the spinal cord I Influence on transmission from primary afferents Acta physiol scand 1966 67 373 386

ARAKI, T J C ECCLES and M ITO Correlation of the inhibitory post-synaptic potential of motoneurones with the latency and time course of inhibition of monosynaptic reflexes J Physiol (Lond) 1960 154 354-377

ARSHAVSKY Y I M B BERKINBLIT O I FUKSON I M GELFAND and G N ORLOVSKY Origin of modulation in neurones of the ventral spinocerebellar tract during locomotion Brain Res 1972 a 43 276-279

ARSHAVSKY Y I M B BERKINBLIT I M GELFAND G N ORLOVSKY and O I FUKSON Activity of neurons of the ventral spino-cerebellar tract during locomotion (In Russian) Biofizika 1972 b 17 883-890

ARSHAVSKY Y I M B BERKINBLIT I M GELFAND G N ORLOVSKY and O I FUKSON Activity of neurons of the ventral spino-cerebellar tract during locomotion of cats with deafferented hindlimbs (In Russian) Biofizika 197_ c 17 1112 1116

BALDISSERA, F and G ten BRUGGENCATE Rubrospinal effects on spinal border cells Acta physiol scand 1969 Suppl 330 119

BALDISSERA, F and F WEIGHT Descending monosynaptic connexions to spinal border cells Acta physiol scand. 1969 76 28A 29A

BERGMANS J R BURKE and A LUNDBERG Inhibition of trans mission in the recurrent inhibitory pathway to motoneurones Brain Res 1969 13 600-602

BRADLEY K and J C ECCLES, Analysis of the fast afferent impulses from thigh muscles J Physiol (Lond) 1953 122 46 -473

BROWN M C D G LAWRENCE and P B C MATTHEWS Antidromic inhibition of presumed fusimotor neurones by repetitive nerve stimu lation of the ventral root in the decerebrate cat Experientia 1968 24 1210-1211

BRUGGENCATE G ten, R BURKE A LUNDBERG and M UDO Interaction between the vestibulospinal tract, contralateral flexor reflex afferents and Ia afferents Brain Res 1969 14 529-532

BURKE R A LUNDBERG and F WEIGHT Spinal border cell origin of the ventral spinocerebellar tract Exp Brain Res 1971 12 283 294

COOPER, S and C S SHERRINGTON Gower s tract and spinal border cells Brain 1940 63 123-134

ELLAWAY P H Antidromic inhibition of fusimotor neurones J Physiol (Lond) 1968 198 39–40P

ELLAWAY P H Recurrent inhibition of fusimotor neurones exhibiting background discharges in the decerebrate and the spinal cat J Physiol (Lond) 1971 216 419–439

ENGBERG I and A LUNDBERG An electromyographic analysis of muscular activity in the hindlimb of the cat during unrestrained locomotion Acta physiol scand 1969 75 614–630

FEDINA L and H HULTBORN Facilitation from ipsilateral primary afferent of interneuronal transmission in the Ia inhibitory pathway to motoneurones Acta physiol scand. 1972 86 59 81

FRANK K and M G F FUORTES Unitary activity of spinal interneurones of cats J Physiol (Lond) 1956 131 424–435

GRANIT R Receptors and Sensory Perception Yale University Press New Haven 1955 1–369

GRANIT R The Basis of Motor Control Academic Press London and New York 1970 1–346

GRILLNER S Supraspinal and segmental control of static and dynamic γ-motoneurones in the cat Acta physiol scand 1969 Suppl 327 1–34

GRILLNER S and M UDO Recruitment in the tonic stretch reflex. Acta physiol scand 1971 81 571–573

HAGBARTH K -E and Å B VALLBO Discharge characteristics of human muscle afferents during muscle stretch and contraction Exp Neurol 1968 22 674–694

HAGBARTH K -E and Å B VALLBO Single unit recordings from muscle nerves in human subjects Acta physiol scand. 1969 76 321–334

HOLMQVIST B and A LUNDBERG Differential supraspinal control of synaptic actions evoked by volleys in the flexion reflex afferents in alpha motoneurones Acta physiol scand 1961 54 Suppl 186 1 51

HONGO T E JANKOWSKA and A LUNDBERG Convergence of excitatory and inhibitory action on interneurones in the lumbosacral cord Exp Brain Res 1966 1 338–358

HONGO T E JANKOWSKA and A LUNDBERG The rubrospinal tract II Facilitation of interneuronal transmission in reflex paths to motoneurones Exp Brain Res 1969 7 365 391

HONGO T E JANKOWSKA and A LUNDBERG The rubrospinal tract IV Effects on interneurones Exp Brain Res 1972 15 54 78

HUBBARD J I and O OSCARSSON Localization of the cell bodies of the ventral spino-cerebellar tract in lumbar segments of the cat J comp Neurol 1962 118 199–204

HULTBORN H Convergence on interneurones in the reciprocal Ia inhibitory pathway to motoneurones Acta physiol scand 197_ a Suppl 375 1-42
HULTBORN H On the control of transmission in the reciprocal Ia inhibitory pathway to motoneurones In Mechanisms of Neuronal Integration in Nerve Centers Kiev 1972 b
HULTBORN H E JANKOWSKA and S LINDSTRÖM Recurrent inhibition of interneurones monosynaptically activated from group Ia afferents J Physiol (Lond) 19 1 a 215 613-636
HULTBORN H E JANKOWSKA and S LINDSTRÖM Relative contribution from different nerves to recurrent depression of Ia IPSPs in motoneurones J Physiol (Lond) 1971 b 215 637-664
HULTBORN H E JANKOWSKA S LINDSTRÖM and W ROBERTS Neuronal pathway of the recurrent facilitation of motoneurones J Physiol (Lond) 1971 c 218 495-514
HULTBORN H and A LUNDBERG Reciprocal inhibition during the stretch reflex Acta physiol scand 1972 85 136-138
HULTBORN H and M SANTINI, Supraspinal control of monosynaptically activated group Ia interneurones in the ventral horn Acta physiol scand 1972 84 142-144
HULTBORN H and M UDO Convergence in the reciprocal Ia inhibitory pathway of excitation from descending pathways and inhibition from motor axon collaterals Acta physiol scand 1972 a 84 95 108
HULTBORN H and M UDO Convergence of large muscle spindle (Ia) afferents at interneuronal level in the reciprocal Ia inhibitory pathway to motoneurones Acta physiol scand 1972 b 84 493-499
HULTBORN H and M UDO Recurrent depression from motor axon collaterals of supraspinal inhibition in motoneurones Acta physiol scand 1972 c 85 44-57
JANKOWSKA E M G M JUKES, S LUND and A LUNDBERG The effect of DOPA on the spinal cord 5 Reciprocal organization of pathways transmitting excitatory action to alpha motoneurones of flexors and extensors Acta physiol scand 1967 70 369 388
JANKOWSKA, E and S LINDSTRÖM Morphology of interneurones mediating Ia reciprocal inhibition of motoneurones in the spinal cord of the cat J Physiol (Lond) 1972 226 805-823
JANKOWSKA, E and S LINDSTRÖM Procion Yellow staining of functionally identified interneurones in the spinal cord of the cat In Intracellular Staining Techniques in Neurobiology Ed S B Kater and C Nicholson Springer Verlag Berlin-Göttingen Heidelberg New York 1973
JANKOWSKA, E A LUNDBERG and D STUART Propriospinal control of last order interneurones of spinal reflex pathways in the cat Brain Res 1973 In press

JANKOWSKA E and W J ROBERTS An electrophysiological demonstration of the axonal projections of single spinal interneurones in the cat J Physiol (Lond) 1972 a 222 597-622
JANKOWSKA, E and W J ROBERTS Synaptic actions of single interneurones mediating reciprocal Ia inhibition of motoneurones J Physiol (Lond) 1972 b 222 623-642
JANSEN J and A BRODAL, Handbuch der mikroskopischen Anatomie des Menschen, IV/8 Das Kleinhirn Springer Verlag Berlin 1958
LAPORTE Y and P BESSOU Etude des sousgroupes lent et rapide du groupe I (fibres afférentes d origine musculaire de grand diamètre) chez le chat J Physiol (Paris) 1957 49 1025 1037
LIDDELL E G T and C S SHERRINGTON Reflexes in response to stretch Proc Roy Soc 1924 96B 212 24.
LINDSTRÖM S and M TAKATA, Monosynaptic excitation of dorsal spinocerebellar tract neurones from low threshold joint afferents Acta physiol scand 1972 84 430-432
LINDSTRÖM S and M TAKATA, Lack of recurrent depression from motor axon collaterals of Ia IPSPs in DSCT cells 1973 To be publ
LLOYD D P C A direct central inhibitory action of dromically conducted impulses J Neurophysiol 1941 4 184 190
LLOYD D P C Reflex action in relation to pattern and peripheral source of afferent stimulation J Neurophysiol 1943 a 6 111 120
LLOYD D P C Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat J Neurophysiol. 1943 b 6 293 315
LLOYD D P C Conduction and synaptic transmission of the reflex response to stretch in spinal cats J Neurophysiol 1943 c 6 317-326
LLOYD D P C Facilitation and inhibition of spinal motoneurons J Neurophysiol 1946 a 9 421-438
LLOYD D P C *Integrative pattern of excitation and inhibition in two-neuron reflex arcs* J Neurophysiol. 1946 b 9 439-444
LUNDBERG A Integrative significance of patterns of connections made by muscle afferents in the spinal cord Symp XXI Int physiol Congr Buenos Aires 1959 1-5
LUNDBERG A Supraspinal control of transmission in reflex paths to motoneurones and primary afferents In Physiology of Spinal Neurons Ed J C Eccles and J P Schadé Elsevier Publ Co Amsterdam Progress in Brain Res 1964 12 197-219
LUNDBERG A Integration in the reflex pathway In Muscular Afferents and Motor Control Nobel Symposium I Ed R Granit *Almqvist & Wiksell*, Stockholm 1966 275-305
LUNDBERG A Convergence of excitatory and inhibitory action on interneurones in the spinal cord In The Interneuron Ed M A B Brazier UCLA Forum Med Sci No 11 University of *California* Press Los Angeles 1969 a 231-265
LUNDBERG A Reflex control of stepping The Nansen Memorial Lecture V Universitetsforlaget, Oslo 1969 b 1-42

LUNDBERG A The excitatory control of the Ia inhibitory pathway In *Excitatory Synaptic Mechanisms* Ed P Andersen and J K S Jansen Universitetsforlaget Oslo 1970 333-340

LUNDBERG A Function of the ventral spinocerebellar tract - a new hypothesis Exp Brain Res 1971 12 317 330

LUNDBERG A and O OSCARSSON Functional organization of the dorsal spino-cerebellar tract in the cat VII Identification of units by antidromic activation from the cerebellar cortex with recognition of five functional subdivisions Acta physiol scand 1960 50 356-374

LUNDBERG A and O OSCARSSON Functional organization of the ventral spino-cerebellar tract in the cat IV Identification of units by antidromic activation from the cerebellar cortex. Acta physiol scand 1962 54 252 269

LUNDBERG A and F WEIGHT Signalling of reciprocal Ia inhibition by the ventral spinocerebellar tract Brain Res 1970 23 109 111

LUNDBERG A and F WEIGHT Functional organization of connexions to the ventral spinocerebellar tract Exp Brain Res 1971 12 295-316

MATTHEWS P B C Mammalian Muscle Receptors and their Central Actions Edward Arnold (Publishers) Ltd London 1972 1-630

MERTON P A Speculations on the servo-control of movements In The Spinal Cord Ed Wolstenholme Churchill Ltd London 1953

MILLER S and O OSCARSSON Termination and functional organiza tion of spino-olivocerebellar paths In The Cerebellum in Health and Disease Ed. W S Fields and W D Willis Jr Warren H Green Inc St. Louis 1970 172 200

NOTH J Recurrente Hemmung der Extensor-Fusimotoneurone? Pflügers Arch 1971 329 23 33

OSCARSSON O Functional organization of the ventral spino-cerebellar tract in the cat I Electrophysiological identification of the tract Acta physiol scand 1956 38 145-165

OSCARSSON O Functional organization of the ventral spino-cerebellar tract in the cat II Connections with muscle, joint, and skin nerve afferents and effects on adequate stimulation of various receptors Acta physiol scand 1957 42 Suppl 146 1-107

OSCARSSON O Functional organization of the ventral spino-cerebellar tract in the cat III Supraspinal control of VSCT units of I type Acta physiol scand 1960 49 171-183

OSCARSSON O Integrative organization of the rostral spinocero bellar tract in the cat Acta physiol scand 1965a 64 154 166

OSCARSSON O Functional organization of the spinocerebellar tracts Physiol Rev 1965 b 45 495-522

OSCARSSON O Functional significance of information channels from the spinal cord to the cerebellum In *Neurophysiological Basis of Normal and Abnormal Motor Activities 3rd Symposium of the* Parkinson s Disease Information and Research Center Ed M D Yahr and D P Purpura Raven Press Hewlett, N Y 1967 93-117

OSCARSSON O Termination and functional organization of the ventral spino-olivocerebellar path J Physiol (Lond) 1968 196 453-478

OSCARSSON O and N UDDENBERG Identification of a spinocerebellar tract activated from forelimb afferents in the cat Acta physiol. scand 1964 62 125-136

OSCARSSON O and N UDDENBERG *Properties of afferent connec-*tions to the rostral spinocerebellar tract in the cat Acta physiol scand 1965 64 143-153

PALKOVITS, M P MAGYAR and J SZENTAGOTHAI, Quantitative histological analysis of the cerebellar cortex in the cat IV Mossy fiber Purkinje cell numerical transfer Brain Res 1972 45 15 29

RENSHAW B Influence of the discharge of motoneurons upon excitation of neighboring motoneurons J Neurophysiol 1941 4 167 183

RENSHAW B Central *effects of centripetal impulses in axons of* spinal ventral roots J Neurophysiol 1946 9 191 204

RYALL, R W Renshaw cell mediated inhibition of Renshaw cells Patterns of excitation and inhibition from impulses in motor axon collaterals J Neurophysiol 1970 33 257 270

RYALL, R W and M F PIERCEY Excitation and inhibition of Renshaw cells by impulses in peripheral afferent nerve fibers J Neuro physiol. 1971 34 242-251

RYALL R W M F PIERCEY and C POLOSA Intersegmental and intrasegmental distribution of mutual inhibition of Renshaw cells J Neurophysiol 1971 34 700 707

RYALL, R W *M F PIERCEY C POLOSA and J GOLDFARB* Excitation of Renshaw cells in relation to orthodromic and antidromic excitation of motoneurons J Neurophysiol 1972 35 137-148

SEARS, T A Efferent discharges in alpha and fusimotor fibres of intercostal nerves of the cat J Physiol (Lond) 1964 174 295-315

SEVERIN F V G N ORLOVSKY and M L SHIK Work of the muscle receptors *during controlled locomotion* Biophysics 1967 12 575 586 (Eng trans of Biofizika 1967 12 502-511)

SEVERIN F V G N ORLOVSKY and M L SHIK Recurrent inhibitory effects on single motoneurones during an *electrically evoked loco-*motion (In Russian) Bull exp Biologii in MedicIny 1968 66 3 9

SEVERIN F V M L SHIK and G N ORLOVSKY Work of the muscles and single motor neurones during controlled locomotion *Biofizika* 1967 12 762 772

SHIK, M L G N ORLOVSKY and F V SEVERIN Organization of locomotor synergism Biophysics 1966 11 1011 1019 (Engl transl of Biofizika 1966 11 879–886)

SMITH, M C The anatomy of the spino-cerebellar fibres in man. I The course of the fibres in the spinal cord and brain stem. J comp Neurol 1957 108 285–352

SPRAGUE J M Spinal 'border cells' and their role in postural mechanism (Schiff-Sherrington phenomenon) J Neurophysiol 1953 16 464–474

TAYLOR, A and M R DAVEY Behaviour of jaw muscle stretch receptors during active and passive movements in the cat. [illegible] 1968 220 301–30

VALLBO Å Muscle spindle response at the onset of [illegible] voluntary contractions in man Time difference between [illegible] and skeletomotor effects J Physiol (Lond.) [illegible]

WILSON V J Recurrent facilitation of spinal reflexes. [illegible] Physiol 1959 42 703 713

WILSON V J and P R BURGESS Disinhibition in the [illegible] J Neurophysiol 1962 a 25 392–404

WILSON V J and P R BURGESS Effects of [illegible] on some motoneurons and interneurons J Neurophysiol [illegible] 25 636–650

WILSON V J F P J DIECKE and W H TALBOT [illegible] toxin on conditioning of spinal motoneurons [illegible] 23 659–666

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 393

From the Department of Physiology Karolinska Institutet
S-104 01 Stockholm Sweden

Reflex Organization and Contraction Properties of Facial Muscles

An experimental study in the cat

By

CHRISTER LINDQUIST

Stockholm 1973

Stockholm 1973
Kungl. Boktryckeriet P.A. Norstedt & Söner
733223

Reflex organization and contraction properties of facial muscles

This review is based on investigations described in the following papers which are being referred to by their Roman numerals

I Mechanisms involved in the cat's blink reflex. *Acta physiol scand* 1970 *80* 149—159 (together with A. Mårtensson)

II Facilitation and inhibition of facial reflexes in the cat induced by peripheral stimulation *Acta physiol. scand* 1972 *85* 126—135

III Analysis of facial reflex facilitation and inhibition by microelectrode recording from the brain stem. *Acta physiol. scand* 1972 *85* 183—192

IV Contraction properties of cat facial muscles. *Acta physiol. scand* In press.

V Histochemical fiber composition of some facial muscles in the cat in relation to their contraction properties. *Acta physiol. scand* In press. (together with L. Edström)

In the review of the individual papers some recent reports by other investigators which were not available when the original papers were published will also be considered.

Before summarizing the results of the individual papers a more detailed background for the experiments not made room for in the original papers will be presented in the Introduction.

Introduction

As compared with the extensive work on reflex control and physiology of extremity muscles very little interest has been taken in the reflex organization and contraction properties of facial muscles. Our knowledge of facial reflexes is based almost exclusively upon clinical observations, whereas there are few experimental studies on animals. Considering that the human facial reflexes have been incorporated into the routine examination of the nervous system it should be desirable to know more about the peripheral origin and the control of facial reflexes. There is also very scanty information on the contraction properties of the facial muscles proper and nothing seems to be known about their relationship to the histochemical fiber composition. These problems can most easily be tackled in animal experiments and the cat was chosen for this purpose in the experiments to be summarized below. Comparisons of results obtained from experiments on cats and man seem to be justified also from a phylogenetic point of view according to the work of Huber and Hughson (1926). They showed that the facial muscles of mammals are derived from two primitive muscle layers the platysma and the sphincter colli profundus. From these primitive muscles there has been a differentiation and specialization along different lines within various groups of mammals. However the muscles studied in the present thesis the orbicularis oculi, the orbicularis oris, the quadratus labii superioris and the caninus muscles are all derived from the sphincter colli profundus and may be identified both in cat and man

Reflex contractions of human facial muscles were described in the late nineteenth century. Reflex blinking in response to a tap over the supraorbital region of the face was noted by Overend (1896) and Escherich (1898) showed that reflex contractions of the lip musculature could be produced by a tap on the upper lip. These reflexes were subsequently studied by a number of clinicians and described under various names according to the presumed origin of the reflex afferents or by the name of the discoverer. Overend interpreted the blink in response to a tap as a true skin reflex but Wartenberg (1945) refuted the concept that it should be a defense reaction and proposed that it might be set up by stretch activation of intramuscular receptors. He called it a myotatic reflex for which he suggested the name the orbicularis oculi reflex. Thus far the ideas advanced regarding the origin of facial reflexes in man had been based on purely clinical observations but in the early fifties Kugelberg and coworkers (Kugelberg 1952, Ekbom, Jernelius and Kugelberg 1952)

approached the problem by means of electromyographic analysis. They were able to show that tap-elicited reflex contractions of the human orbicularis oculi as well as of perioral muscles are actually composed of two different reflex discharges, one of short latency and one of longer latency. They arrived at the conclusion that the early reflex components were monosynaptic "myotatic" reflexes and the late components nociceptive reflex responses. This seemed to reconcile the divergent opinions prevailing as to the nature of the reflexes. However Kugelberg himself drew attention to the fact that muscle spindles had not been found in facial muscles of man or other mammals. This uncertainty still prevailing as to the peripheral origin of the facial reflexes initiated the experiments presented in paper I.

As early as in Overend's work it had been suggested that the afferent link of the blink reflex was in the primary division of the trigeminal nerve. By electrical stimulation of trigeminal afferents Kugelberg (1952) could show that this applies to both reflex components. That afferents in the trigeminal nerve are involved in facial reflexes is now well established. However some observations indicate that afferents in the facial nerve may also play a part in the elicitation of the early (Rushworth 1962) as well as the late component (Gandiglio and Fra 1967) of the human blink reflex. From histological studies of the cat's facial nerve we know that a small percentage of its thin myelinated afferent nerve fibers take their origin in the mimic muscles (Bruesch 1944). Their possible role in the reflex control of the facial muscles is also considered in paper I.

The animal experiments performed on the facial reflexes are chiefly those of Tokunaga and coworkers (Tokunaga, Oka, Murao, Yokio, Okumura, Hirata, Miyashita and Yoshitatsu 1958) who took up investigations on the central pathways of the double trigemino-facial reflexes. They showed that the early reflex component was absent following lesions of the ipsilateral trigeminal main sensory nucleus and that its threshold was lowered when the contralateral half of the midbrain was transected. The long latency reflex component was absent following the latter procedure and also following lesions to the contralateral ventral thalamic nucleus. Ipsilateral trigeminal tractotomy or destruction of the trigeminal main sensory nucleus raised the threshold for but did not abolish the late reflex component. The conclusion was drawn that the respective reflex pathways pass through the regions where lesions cause a decrement of the respective reflex. The possibility was not considered that systems having a facilitatory or inhibitory influence on the reflex chains may have been damaged. However since the work of Tokunaga and coworkers was presented a number of studies of the sensory impulse flow in trigeminal afferents have been published. It has been shown that there is a control of the trigeminal afferent inflow from suprabulbar as well as from peripheral sources (for review see *e.g.* Darian-Smith 1966). The importance of this sensory gate for the control of facial reflex activity has not been investigated. The interaction between various peripheral sources in the

control of facial muscle reflex activity is therefore considered in paper II and further analyzed in paper III

But little attention has been given to the contraction properties of the facial muscles only single observations of contraction times in man have been reported. However it may be of interest to recollect that the Swedish histologist Häggqvist (1940) undertook studies of the efferent innervation of the facial muscles from which he made implications concerning their physiology He found that the facial nerve in rhesus monkeys and cats is made up of small-diameter nerve fibers. He also identified motor nerve endings of the type later shown to be typical of slow tonic non-twitch muscle fibers, but this finding could not be confirmed in a recent work by Hess (1962) The small motoneurons in the spinal cord seemed specifically concerned with the maintenance of skeletal muscle tone and consequently the facial muscles should have "a pronounced tonic mode of working (Häggqvist 1940) Even though it has later been shown that the small motoneurons of the spinal cord have the lowest threshold for recruitment in the stretch reflex (Henneman, Somjen and Carpenter 1965) it is not a corollary that the same relationship between nerve morphology and muscle function applies to the facial muscles. An answer to this question can only be given by studying the contraction properties of the facial muscles, and results from such experiments are given in paper IV

Ever since Ranvier in the late nineteenth century observed that there are functional differences between "red" and "white muscles the possibilities of correlating structure biochemistry and muscle function have considerably increased In particular with the advent of histochemical techniques it has become possible to correlate characteristics in metabolism of a muscle fiber to certain contraction properties. The availability of such methods is utilized in paper V in an attempt to form a more coherent picture of the facial muscle reflex system.

Results and Discussions

Paper I. Mechanisms involved in the cat's blink reflex

This paper deals with four specific questions: 1) does tap stimulation of the cat's face give rise to reflexes similar to those evoked in man? 2) how is the double facial muscle reflex discharge produced? 3) are muscle spindles engaged in the reflex control of facial muscles or do other types of end-organs serve this purpose? 4) do facial nerve afferents play a part in the reflex control of facial muscles?

The first approach to these problems was obviously to create an experimental situation similar to that used by Kugelberg in his human experiments. Thus, tap stimulations were applied to different facial areas and the EMG led off from the orbicularis oculi and the perioral muscles. Particular attention was given to the reflex discharges of the orbicularis oculi since the most constant responses were recorded from this muscle, and it was found that also in the cat a double reflex discharge results on tap stimulation of the facial skin. The early response had a latency range of 9—12 ms and the late component a range of 15—25 ms. As in the human experiments the reflex responses evoked on tapping at sites remote from the eye were weaker than those evoked from sites close to the eye. There were some observations that did not seem to fit in with the concept that the early reflex component was generated by activation of muscle spindles. Its latency variations were greater than should be expected from a monosynaptic reflex, it was very frequency-sensitive, it had a fairly long duration and, above all, it could be evoked by tapping a skin flap dissected free from the underlying muscle but not by tapping the muscle proper. The multisynaptic origin of the early reflex component was supported by estimates of its central relay time which gave values of 4 ms. The mechanisms responsible for the late component were also studied and the conclusion was drawn that there is a separate reflex chain but that peripheral mechanisms may be operative in its potentiation. Thus, an off volley may be set up by reactivation of skin receptors when the stimulus probe is withdrawn from the skin and if the stimulus is brief enough this off volley may, via a short latency reflex transmission, occur at the moment when the long latency reflex could be expected. Furthermore, skin receptors may be activated by the early reflex contraction and thus reactivate the short latency reflex chain, resulting in a double reflex discharge.

Recently Shahani (1970) and Shahani and Young (1972) reinvestigated the human orbicularis oculi reflex and arrived at the conclusion that also in man the two components of the reflex are elicited by activation of mechanoreceptors in the facial skin.

Although it proved impossible to evoke monosynaptic facial reflexes, attempts were made to record afferent impulses from muscle spindles or other intramuscular receptors. These attempts were unsuccessful even though succinylcholine was used as a potent stimulator of intrafusal muscle fibers. There was thus further reason for the opinion that the facial muscles lack a conventional proprioceptive reflex control system. However it was found that even a very small muscle contraction may give rise to a marked reflex contraction by activation of skin receptors discharging through trigeminal nerve afferents. It was suggested that such afferent discharges may be of some importance for the proprioceptive control of phasic facial muscle contractions. This concept has gained support from recent experiments on human subjects with and without local anesthetic block of trigeminal afferents (Leanderson and Persson 1972)

Weak electrical stimulation of the peripheral facial nerve branches elicited reflexes in various facial muscles. These reflexes disappeared on section of the appropriate trigeminal nerve branches and could not be recorded from the facial nerve in curarized cats. Thus, no evidence for the presence of large-diameter afferents in the facial nerve was found. However when a stronger electrical stimulation was applied to the ventral branch of the facial nerve a reflex discharge with a latency of 25 ms could be recorded in the nerve to the orbicularis oculi even in the curarized cat. The threshold for these reflexes was around six times that of maximal activation of the facial motor nerve fibers, and it was therefore concluded that these reflexes must have been set up by activation of the thin myelinated facial nerve afferents (cf above) The reflexes were not identical with the tap-elicited facial reflexes since no afferent discharge could be recorded in the facial nerve in response to tap stimulation.

Paper II. Facilitation and inhibition of facial reflexes in the cat induced by peripheral stimulation

In this paper the effects of conditioning stimuli on the reflexes studied in paper I are described and analyzed. (A further analysis of the conditioning effects is given in paper III) Reflexes set up in the orbicularis oculi and caninus muscles by tap stimulation of the face were subjected to conditioning stimuli of various types. When the conditioning stimulus was a facial tap reflex facilitation resulted when the conditioning test interval was less than 20 ms, whereas with intervals between 40 and 80 ms reflexes of lower amplitude were obtained. Reflex inhibition was observed even with conditioning test intervals up to 450 ms. A reflex depression was also noted when the conditioning tap was given to contralateral areas. It was therefore unlikely that the inhibitory effects were due to a skin deformation persisting from the conditioning facial tap This could also be ruled out by the fact that facilitation

as well as inhibition of facial reflexes could be obtained also by an electrical conditioning stimulation of trigeminal nerve afferents. It was concluded that central nervous mechanisms were involved in the interaction phenomena. It was also found that electrical stimulation of afferents in various other peripheral nerves could condition the tap-elicited reflexes. Thus electrical stimulation of lingual and hypoglossal nerve afferents resulted in periods of facilitation and inhibition whereas inhibition invariably resulted on conditioning by stimulating afferents in the ipsilateral radial or saphenous nerves.

The time course of the inhibitory effects suggested presynaptic inhibition. To test whether pre- or postsynaptic inhibition was involved, drugs were injected for selective blocking of one or the other type of inhibition, picrotoxin being used to block the presynaptic and strychnine to block the postsynaptic type. These experiments showed a clear difference between the inhibitory effect elicited by conditioning stimuli from distant sites such as radial nerve afferents and inhibition induced by facial conditioning taps, in that the latter but not the former was diminished by strychnine. The effects of picrotoxin were equivocal. The conclusion was drawn that postsynaptic inhibition is involved when the conditioning stimulus is a tap to the facial skin The reflex depression evoked from distant sites was considered to be due to presynaptic inhibition because of its longer duration and its resistance to strychnine. Presynaptic inhibition was a likely explanation also for the late part of the long-lasting depression induced by conditioning facial taps.

In man, habituation of the blink reflex results on repeated stimulation. A small series of experiments was made to find out whether this is the case also in the anesthetized cat and if possible to find an explanation for the phenomenon. The tap-elicited blink reflex was found to be considerably depressed even at a frequency of 4/s which agrees well with the findings in man. Since habituation occurred also in decerebrate cats, cortical mechanisms, which have been widely accepted as the cause of blink reflex habituation in man, could be ruled out. Instead it was suggested that presynaptic inhibition at the brain stem level should be involved since the interval between the taps at the lowest stimulus frequency producing habituation was approximately similar to the longest interval at which the test reflexes were depressed in the conditioning test experiments.

Paper III. Analysis of facial reflex facilitation and inhibition by microelectrode recording from the brain stem

In this paper a further step in the analysis of the reflex control of facial muscles is taken by recording events in the facial motor nucleus with extracellular microelectrodes. The compound antidromic response resulting from stimulation of various peripheral facial nerve branches is described The effect on these potentials of

conditioning antidromic facial nerve volleys was then studied with the particular view in mind to elucidate whether the motor impulse flow from facial motoneurons like that from the spinal motoneurons is controlled by an inhibitory (or facilitatory) feed back mechanism from motor axon collaterals (Renshaw inhibition) It was found that the excitability of the motoneuron pool was regularly depressed for 30 ms and sometimes even for up to 100 ms following antidromic invasion. In contrast to *the Renshaw type of inhibition at the spinal level this decreased excitability* was unaffected by injections of strychnine. It was therefore concluded that it was due to post-spike hyperpolarization of motoneurons rather than to feed-back inhibition.

In a short communication Kitai and coworkers have reported a similar interaction of antidromic volleys in the facial nucleus of the cat. They also made intracellular recordings from some motoneurons and found a duration of after-spike hyperpolarization *of up to around 30—40 ms. To account for the longer periods of depressed* excitability in the motor nucleus they postulated some inhibitory mechanism. Strychnine was not used in their analysis (Kitai Akaike Bando Tanaka, Tsukahara and Yu 1971)

In paper III the effects on the motoneuron pool of trigeminal synaptic activation are also analyzed in order to see to what extent the facilitatory and inhibitory phenomena described in paper II occur in the facial motoneurons. The field potential evoked in the facial nucleus by electrical stimulation of peripheral trigeminal nerve branches is described. The amplitude of the antidromically elicited potential in the facial nucleus was taken as an index of the current state of excitability of the motoneurons. The results showed that there is an increased motoneuron excitability 3—35 ms following a facial tap stimulation and a period of lowered motoneuron responsiveness from 35 to 65 ms following the facial conditioning tap. The initial period of antidromic potential facilitation was interpreted as being due to exitatory postsynaptic potentials set up by the afferent volley in facial motoneurons and the period of lowered excitability as coincident with a period when facial motoneurons are under postsynaptic inhibitory influence. Further support was thus given to the idea put forward in paper II that postsynaptic inhibition is involved in the control of facial muscle reflex excitability

The trigeminal synaptic input to the facial nucleus has now been recorded intra cellularly from facial motoneurons by Tanaka, Yu and Kitai (1971) *In their study* exitatory postsynaptic potentials on electrical stimulation of peripheral trigeminal nerve branches were found to occur in facial motoneurons with a minimum latency of 2—3 ms, which should correspond to an input by di or polysynaptic pathways. Their paper and the results reported here thus support the findings presented in paper I showing that trigemino-facial reflexes are not monosynaptic.

In paper III discharges from reticular neurons in response to stimulation of facial and hypoglossal nerve afferents are also described. The latencies of the responses of

these neurons and their discharge properties were similar to those of neurons responsible for the N_2 component of the trigeminal field potential and their location in the brain stem similar. The N_2 component is related to depolarization of trigeminal afferent terminals in the brain stem. It was therefore suggested that facial and hypoglossal nerve afferents may exert a depolarizing action on afferent trigeminal brain stem terminals by these reticular neurons and thus produce presynaptic inhibition of facial reflexes.

Paper IV. Contraction properties of cat facial muscles

Facial muscles have been studied from various aspects but very little attention has been paid to the physiological characteristics of the effector organs, *i.e.* to the contraction properties of the muscles. Values of contraction times occasionally recorded in, *e.g.* the human frontalis muscle (McComas and Thomas 1968) indicate that they are fast. As mentioned in the introduction it has been inferred from histological studies that the facial muscles have a tonic mode of functioning which is usually characteristic of the slow "red" striated muscles. Such a mode of action is however inconsistent with the morphology of the facial muscles in which no muscle spindles could be identified in the electrophysiological experiments described in paper I or in the histological studies presented in paper V. In fact, it is difficult to set up a tonic discharge in facial muscles even in a decerebrate cat (unpublished observation). The experiments described in paper IV were undertaken to study the contraction properties of the facial muscles, the main interest being focused on the orbicularis oculi and oris. These muscles were chosen because minimal surgery is required and the risk of injury to the superficial and delicate muscle fibers thus small. A comparison of the contraction properties of the two muscles is of interest also because they differ functionally, the orbicularis oculi having, in contrast to the orbicularis oris, a very low threshold for reflex activation (cf. paper I).

Maximal isometric contractions in response to motor nerve stimulation were recorded from the orbicularis oculi and oris as well as from the depressor conchae. The average contraction times were found to be 8.5 ms, 33 ms and 24 ms respectively. The three muscles may thus all be classified as fast twitch muscles. When electrical stimuli of increasing strength, from threshold to supramaximal values, were applied to the motor nerves of the orbicularis oculi and oris, no appreciable differences could be observed in the time course of the resulting twitches. The *motor units* in the respective muscles should thus have uniform twitch contraction characteristics.

The responses of the orbicularis oculi and oris to repetitive nerve stimulation were studied as well. Summation of individual twitches was found to start at a frequency around 20/s in the former muscle but in the latter at a frequency as low as around

conditioning antidromic facial nerve volleys was then studied with the particular view in mind to elucidate whether the motor impulse flow from facial motoneurons like that from the spinal motoneurons is controlled by an inhibitory (or facilitatory) feed-back mechanism from motor axon collaterals (Renshaw inhibition) It was found that the excitability of the motoneuron pool was regularly depressed for 30 ms and sometimes even for up to 100 ms following antidromic invasion. In contrast to the Renshaw type of inhibition at the spinal level this decreased excitability was unaffected by injections of strychnine. It was therefore concluded that it was due to post-spike hyperpolarization of motoneurons rather than to feed-back inhibition.

In a short communication Kitai and coworkers have reported a similar interaction of antidromic volleys in the facial nucleus of the cat. They also made intracellular recordings from some motoneurons and found a duration of after-spike hyperpolarization of up to around 30—40 ms. To account for the longer periods of depressed excitability in the motor nucleus they postulated some inhibitory mechanism Strychnine was not used in their analysis (Kitai Akaike Bando Tanaka, Tsukahara and Yu 1971)

In paper III the effects on the motoneuron pool of trigeminal synaptic activation are also analyzed in order to see to what extent the facilitatory and inhibitory phenomena described in paper II occur in the facial motoneurons. The field potential evoked in the facial nucleus by electrical stimulation of peripheral trigeminal nerve branches is described. The amplitude of the antidromically elicited potential in the facial nucleus was taken as an index of the current state of excitability of the motoneurons. The results showed that there is an increased motoneuron excitability 5—35 ms following a facial tap stimulation and a period of lowered motoneuron responsiveness from 35 to 65 ms following the facial conditioning tap. The initial period of antidromic potential facilitation was interpreted as being due to exitatory postsynaptic potentials set up by the afferent volley in facial motoneurons and the period of lowered excitability as coincident with a period when facial motoneurons are under postsynaptic inhibitory influence. Further support was thus given to the idea put forward in paper II that postsynaptic inhibition is involved in the control of facial muscle reflex excitability

The trigeminal synaptic input to the facial nucleus has now been recorded intracellularly from facial motoneurons by Tanaka, Yu and Kitai (1971) In their study exitatory postsynaptic potentials on electrical stimulation of peripheral trigeminal nerve branches were found to occur in facial motoneurons with a minimum latency of 2—3 ms, which should correspond to an input by di- or polysynaptic pathways. Their paper and the results reported here thus support the findings presented in paper I showing that trigemino-facial reflexes are not monosynaptic.

In paper III discharges from reticular neurons in response to stimulation of facial and hypoglossal nerve afferents are also described. The latencies of the responses of

these neurons and their discharge properties were similar to those of neurons responsible for the N_2 component of the trigeminal field potential and their location in the brain stem similar. The N_2 component is related to depolarization of trigeminal afferent terminals in the brain stem. It was therefore suggested that facial and hypoglossal nerve afferents may exert a depolarizing action on afferent trigeminal brain stem terminals by there reticular neurons and thus produce presynaptic inhibition of facial reflexes.

Paper IV Contraction properties of cat facial muscles

Facial muscles have been studied from various aspects but very little attention has been paid to the physiological characteristics of the effector organs, i.e. to the contraction properties of the muscles. Values of contraction times occasionally recorded in, e.g. the human frontalis muscle (McComas and Thomas 1968) indicate that they are fast. As mentioned in the introduction it has been inferred from histological studies that the facial muscles have a tonic mode of functioning which is usually characteristic of the slow "red" striated muscles. Such a mode of action is however inconsistent with the morphology of the facial muscles in which no muscle spindles could be identified in the electrophysiological experiments described in paper I or in the histological studies presented in paper V. In fact, it is difficult to set up a tonic discharge in facial muscles even in a decerebrate cat (unpublished observations). The experiments described in paper IV were undertaken to study the contraction properties of the facial muscles, the main interest being focused on the orbicularis oculi and oris. These muscles were chosen because minimal surgery is required and the risk of injury to the superficial and delicate muscle fibers thus small. A comparison of the contraction properties of the two muscles is of interest also because they differ functionally, the orbicularis oculi having, in contrast to the orbicularis oris, a very low threshold for reflex activation (cf. paper I).

Maximal isometric contractions in response to motor nerve stimulation were recorded from the orbicularis oculi and oris as well as from the depressor conchae. The average contraction times were found to be 8.5 ms, 33 ms and 24 ms respectively. The three muscles may thus all be classified as fast twitch muscles. When electrical stimuli of increasing strength, from threshold to supramaximal values, were applied to the motor nerves of the orbicularis oculi and oris, no appreciable differences could be observed in the time course of the resulting twitches. The motor units in the respective muscles should thus have uniform twitch contraction characteristics.

The responses of the orbicularis oculi and oris to repetitive nerve stimulation were studied as well. Summation of individual twitches was found to start at a frequency around 20/ in the former muscle but in the latter at a frequency as low as arou

10/s. No further increase in tension was observed in the orbicularis oculi at frequencies above 100/s and in the orbicularis oris above 60/s there is thus a wider working range for the orbicularis oculi The relation between the maximal tetanic contractions and the twitches was 7.0 and 4.5 respectively

In an attempt to find an explanation for the large differences in contraction times between the two muscles, the duration of the maximal intensity of the active state in a single twitch was estimated using three different, well-known techniques (cf paper IV) Nerve stimulation was used Since this does however not give a synchronous activation of the muscle which is needed for exact measurements, it is possible that small differences between the muscles were not disclosed The range of the values found was 3.5 to 5 ms for both muscles differences that might account for the dissimilarities in contraction times were thus not found in these experiments. According to Hill's (1949) generally accepted theory the fall of the active state curve from its maximum passes through the peak of the twitch curve, and since the tetanus/twitch ratios of the two muscles was known, it was suggested that differences in the rate of fall of the active state may be one factor accounting for the different contraction times. The experiments did however not yield any information about the "intrinsic speed of shortening" of the muscles or about the behavior of their series-elastic elements during contraction, which are the other two factors determining the contraction time of a muscle. However a difference in the intrinsic speed of shortening" should be accompanied by differences in the activity of myofibrillar ATPase (cf Close 1972) and no such difference was observed in the histochemical studies presented in paper V

The first component in the human blink reflex does not to any great extent contribute to the downward movement of the eyelid according to some investigators (Shahani and Young 1972) On the other hand, there are findings indicating that one and the same motor unit may discharge both in the short and the long latency blink reflex component (Shahani 1970) In order to seek a clue to these somewhat discrepant findings, the EMG was recorded simultaneously with the actual contraction in a tap-elicited blink reflex. It was then found that a contraction produced by the short latency reflex component alone attains its maximum in around 15 ms and that it may be 2—3 times larger than a maximum twitch These findings indicate that fast motor units discharging repetitively are recruited in the early reflex response. The contraction elicited by a double reflex discharge reached a peak in around 30 ms indicating that the prolongation of the reflex contraction must have been produced by motor units about as fast as the units contracting in the early response since the latency of the second reflex response is 15—25 ms (see paper I) A difference in function between the two reflex components was thus not apparent from the investigations presented in this paper and in paper I It was however observed that a double reflex is most readily evoked on tapping in the close vicinity of the eye

(see paper I) and this may imply that the function of the long latency reflex is to prolong and strengthen the blink reflex when a noxious object threatens the eye

Paper V Histochemical fiber composition of some facial muscles in the cat in relation to their contraction properties

An increasing number of papers are being devoted to studies of enzyme activity and substrate content in skeletal muscle by histochemical techniques since it has proved possible to relate the results of such investigations to functional properties of the muscles. In particular the activity of myofibrillar ATPase seems to be higher in fast muscles and the activity of oxidative enzymes higher in muscles with a well developed capacity for prolonged work.

In the experiments described in paper V muscle biopsies were taken from the orbicularis oculi, the depressor conchae and the orbicularis oris muscles and immediately frozen in liquid nitrogen. The material was then stained by various methods to show the activity of myofibrillar ATPase, the two oxidative enzymes succinic dehydrogenase and $NADH_2$ tetrazolium reductase and the glycolytic enzyme phosphorylase as well as the content of glycogen and fat The three investigated muscles all contained muscle fibers with a high (type II) and such with a low (type I) activity of myofibrillar ATPase. The former type made up 90 % of the fiber population in the orbicularis oculi 85 % of the fibers in the orbicularis oris and 70 % of those in the depressor conchae. Considering the large difference in contraction times between the orbicularis oculi and oris it was surprising to find an almost equal number of fibers with a high activity of myofibrillar ATPase in the two muscles. This could be interpreted in one of two ways either there is no direct correlation between the intrinsic speed of shortening and the activity of myofibrillar ATPase or the differences in contraction time are due to other factors than to dissimilarities in the intrinsic speed of shortening One such factor may be a difference in the decay of the active state, as pointed out in paper IV and this may in turn be due to differences in the rate of uptake of calcium into the sarcoplasmic reticulum, which may be independent of the myofibrillar ATPase activity

The facial muscle fibers with a low activity of myofibrillar ATPase (type I) seem to be equipped for an aerob c type of metabolism. Thus the activity of the oxidative enzymes was high or intermediate, as was also the fat content, whereas the glycogen content and phosphorylase activity was low This conforms to the histochemical characteristics of type I fibers in extremity muscles.

The fibers with a high activity of myofibrillar ATPase (type II) have a high activity of phosphorylase and a high glycogen content, which is typical of such fibers also in extremity muscles There was always a correspondence between the

fat content and the activity of the oxidative enzymes thus, if the fat content in a fiber was low intermediate or high the enzyme activity was also low intermediate or high respectively The predominant type of fiber in this group had an intermediate content of fat and an intermediate activity of oxidative enzymes in the orbicularis oris, but in the other two muscles a low oxidative enzyme activity and a low fat content Thus among the facial muscle fibers with a high activity of myofibrillar ATPase there are those equipped for aerobic and anaerobic metabolism and those designed only for anaerobic metabolism. The latter fibers are white in appearance and the former red.

Since the orbicularis oris is composed mainly of fibers with an intermediate staining reaction for oxidative enzymes it should be expected to perform better than the orbicularis oculi in prolonged work. This was tested by stimulating the motor nerves to the two muscles at 5—20/s for various lengths of time and recording the resulting twitch tension decline In comparison with a prestimulus twitch elicited at 1/s there was on the average a 3[illegible] % loss of twitch tension in the orbicularis oculi after stimulation of the muscle at 5/s for 10 min whereas only 13 % of the twitch tension had been lost in the orbicularis oris. Muscles subjected to such periods of prolonged stimulation were examined histochemically after the experiment and the activity of succinic dehydrogenase was found to be similar to that in an unstimulated muscle, but the glycogen had been depleted from many fibers in the orbicularis oculi. The glycogen depletion was primarily confined to muscle fibers with a low activity of succinic dehydrogenase. Thus, these experiments confirm the previous finding that there is a relationship between glycogen depletion and muscle fatigue and they validate the assumption that the orbicularis oris performs better than the orbicularis oculi during prolonged work.

Papers IV and V show that the facial muscles do not form a functionally homogeneous group but that there is a differentiation of their contraction properties which is accompanied by differences in enzyme activity and substrate content.

References

Bruesch, S. R., The distribution of my linated afferent fibers in the branches of the cat's facial nerve. *J comp Neurol.* 1944 *81* 169—191

Close, R. I Dynamic properties of mammalian skeletal muscles. *Physiol. R v* 1972 *52* 129—197

Darian-Smith I Neural mechanisms of facial sensation. *Int Rev Neurobiol* 1966 *9* 301—395

Ekbom, K. A., B. Jernelius and E. Kugelberg, Perioral reflexes. *Neurology* (Minneap.) 1952. *2* 103—111

Escherich, Traité des maladies de l'enfance. Grancher Comby et Marfan. Paris 1898. vol. 4 p. 730

Gandiglio, G and L. Fra, Further observations on facial reflexes. *J Neurol. Sci.* 1967 *5* 273—285.

Hagbarth, G A contribution to the question of the nervous and muscular substratum of the muscle tone. *A t med. and* 1940. *54* 8—20.

Henneman E., G Somjen and D O Carpenter Functional significance of cell size in spinal motoneurons. *J Neurophysiol.* 1965 *28* 560—580

Hess, A. Further morphological observations of en plaque and en grappe nerve endings on mammalian extrafusal muscle fibers with the cholinesterase technique. *Rev c and Biol.* 1962. *21* 241—248.

Hill, A. V The abrupt transition from rest to activity in muscle. *Proc roy Soc B.* 1949 *136* 399—420.

Huber E. and W Hughson, Experimental studies on the voluntary motor innervation of the facial musculature. *J comp Neurol.* 1926. *42* 113—163

Kitai, S. T T Akaike, T Band T Tanaka, N Tsukahara and H. Yu, Antidromic and synaptic activation of the facial nucleus of cat. *Brain Res* 1971 *33* 227—232

Kugelberg, E., Facial reflexes. *Brain.* 1952. *75* 385—396

Leanderson, R. and A. Persson, The effect of trigeminal nerve block on the articulatory EMG activity of facial muscles. *Act Otolaryng* 1972. *74* 271—278.

McComas, A J and H. C. Thomas, Fast and slow twitch muscles in man. *J Neurol. Sci* 1968 *7* 301—307

Overend, W Preliminary note on a new cranial reflex. *Lancet* 1896. *1* 619

Rushworth, G Observations on blink reflexes. *J Neurol. Neurosurg Psychiat* 1962. *25* 93—108

Shahani B The human blink reflex *J Neurol. Neurosurg Psychiat* 1970 *33* 792—800.

Shahani B. and R. Young, Human orbicularis oculi reflexes. *Neurology* 1972 *22* 149—154

Tanaka, T H. Yu and S. T Kitai, Trigeminal and spinal inputs to the facial nucleus. *Brain Res* 1971 *33* 504—508

Tokunaga, A., M. Oka, T Murao, H. Yokio, T Okumura, T Hirata, Y Miyashita and S. Yoshitatsu An experimental study on facial reflex by evoked electromyography *Med J Osaka Univ* 1958. *9* 397—411

Wartenberg R., The examination of reflexes. The year book publishers. Chicago 1945

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 394

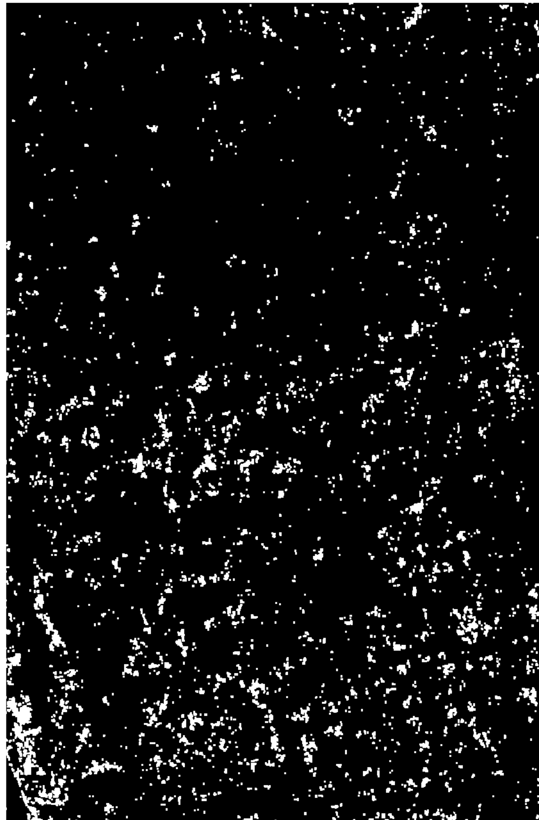
DEVELOPMENT OF SOMATOSENSORY CORTICAL FUNCTIONS

AN ELECTROPHYSIOLOGICAL STUDY IN PRENATAL SHEEP

BY

HANS E. PERSSON

STOCKHOLM 1973



ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 394

FROM THE DEPARTMENT OF PHYSIOLOGY
KAROLINSKA INSTITUTET
STOCKHOLM 60, SWEDEN

DEVELOPMENT OF SOMATOSENSORY CORTICAL FUNCTIONS

AN ELECTROPHYSIOLOGICAL STUDY IN PRENATAL SHEEP

BY

HANS E PERSSON

STOCKHOLM 1973

BOKTRYCKERI AB THULE, STOCKHOLM 1973

CONTENTS

INTRODUCTION 5

METHODS 9

I Electrophysiology 9

1 Material 9

2. Animal preparation 9

3 Stimulation 11

4 Recording 11

5 Methods of laminar potential analysis 12

II Histology 14

RESULTS 15

I Development of somatosensory evoked surface responses 15

1 Cortical distribution 15

2 Configuration 17

3 Latency 18

4 Amplitude 19

5 Repetitive stimulation 20

6 Effect of strychnine 22

7 Afterdischarges 23

II Development of somatosensory evoked field potentials 24

III Development of somatosensory evoked single unit activity 30

1 Response characteristics 30

2. Repetitive stimulation and recovery cycles 33

3 Relation to evoked surface responses 34

4 Depth-distribution 35

5 Inhibition of cortical neurons 37

6 Relation to evoked surface afterdischarges 37

IV Development of cortical morphology 38

DISCUSSION 42

1 Aspects of the mode of operation in the immature somatosensory system 42

2 Development of the specific and nonspecific somatosensory systems 45
3 Functional development of the somatosensory cortex and its morphological correlates 47

SUMMARY 53

ACKNOWLEDGEMENTS 55

REFERENCES 56

INTRODUCTION

The development of functions of sensory systems was originally analysed from the appearance and changes of the reflexes which could be elicited by afferent stimulations (for reviews, see e.g. Windle 1941 Carmichael 1951 1954 Gottlieb 1971) Several basic developmental concepts were postulated such as the functional precocity of somesthesia, particularly the trigeminal afferent inflow compared to audition and vision.

The introduction and use of electrophysiological techniques, notably the evoked potential method made it possible to obtain further information about the functional development of the sensory systems. The investigations on somatosensory evoked cortical responses in newborn dogs, cats, rabbits and rats (Scherrer and Oeconomos 1954 Grossman 1955 Marty 1962 Delhaye Bouchaud 1964 Thairu 1971 Verley and Rokyta 1972) give conclusive evidence that the afferent connections from the skin receptors to the cortical projection areas are already present at birth in these animals. Recent studies on the visual system during postnatal development have provided data on the involvement of different subcortical and cortical structures in the generation of various components of the evoked cortical response (Rose 1968 a,b 1971 Rose and Lindsley 1968) Comparable investigations have not been made on the maturation of somatosensory cortical responses. However the relative functional importance of the afferent pathways directed towards the somesthetic cortex of postnatal animals can be deduced from findings on electrocortical activities in primary and nonprimary projection areas evoked by electrical stimulation of ventrobasal and intralaminar thalamic nuclei and reticular structures (Purpura 1961 a,b, 1962, Scheibel 1962, Scheibel and Scheibel 1964 1971 Verley Siou and Garma 1966, Verley 1967 a, Verley and Siou 1967) In addition, responses have been described in subcortical relay stations evoked by peripheral tactile stimulation (Verley 1967 b)

The importance of the number and speed of the neuronal signals for the functional differences between the neonatal and the mature afferent systems has recently been discussed (Scherrer Verley and Garma 1968, 1970) Interest has also been paid to the ability of the immature sensory cortex to code information concerning different qualities of the peripheral stimuli. Thus,

there are data available on the postnatal development of receptive field characteristics in the visual cortex (Hubel and Wiesel 1963 Wiesel and Hubel 1963 Barlow and Pettigrew 1971) and of somatotopic organization in the somesthetic cortex (Rubel 1971) The results indicate that the functional organization of the cells within the somesthetic cortex, which serve the processing of afferent information is fairly well organized at birth in postnatal cats. No investigation has been directed towards the problem of the building up of this functional organization during the early stages of ontogeny

Several studies on mammals employing natural and electrical stimulation of different peripheral receptors have demonstrated qualitative and quantitative changes in the electrocortical responses as a function of age (Hunt and Goldring 1951 Scherrer and Oeconomos 1954 Rose, Adrian and Santibanez 1957 Ellingson and Wilcott 1960 Marty 1962 Marty and Thomas 1963 Delhaye-Bouchaud 1964 Pujol Granier and Marty 1966 Molliver 1967 Fox 1968 Mysliveček 1968 a, Pujol and Marty 1968 Rose 1968 a,b 1971 Rose and Lindsley 1968 Meyerson and Persson 1969 Pujol 1971 Sedlaček 1971 Thairu 1971 Briquel and Verley 1972 Persson and Stenberg 1972 Rose, Gruenau and Spencer 1972 Verley and Rokyta 1972) In newborn dogs, cats, rabbits and rats stimulation in the periphery of the somatosensory system generates activity in the cortex as represented by a long-latency surface negative response (Scherrer and Oeconomos 1954 Grossman 1955 Marty 1962 Delhaye Bouchaud 1964 Thairu 1971) On the basis of studies on effects of pharmacological agents on gross surface responses in the somatosensory cortex of neonatal kittens it has been suggested that both excitatory and inhibitory synaptic processes are involved at different levels of the cortex (Purpura 1961 c Purpura Shofer Housplan and Noback 1964) The relative importance of inhibitory synaptic mechanisms in the newborn feline cortex has been repeatedly stressed (Purpura, Shofer and Scarff 1965 Purpura 1969 1971) Correlative morphological studies show that the cortical cyto-architectonics at this neonatal stage are already relatively differentiated with well separated layers containing neurons in different phases of maturation (*e.g* Cajal 1960 Noback and Purpura 1961 Marty 1962) In addition, electron microscopic investigations on the cortex of perinatal dog cat and mouse have demonstrated a characteristic abundance of axodendritic synapses in the superficial cortical layers (Voeller Pappas and Purpura 1963 Meller Bleipohl and Clees 1968 Molliver and van der Loos 1970 Adinolfi 1971 1972) The prominent superficial neuropile with well-developed axodendritic synapses has been considered to account for the predominating surface negative evoked and spontaneous electrocortical activities in the neonate (Marty

Chevreau and Scherrer 1961 Marty 1962 Purpura *et al* 1964) During the late postnatal period the somesthetic evoked cortical potential undergoes a change into its adult positive-negative form. The evolvement of basilar dendrites and axosomatic synapses (Marty *et al.* 1961 Marty 1962 Purpura *et al.* 1964) and a deep neuropile with specific corticopetal fibers as well as dendrites and axonal network of interneurons (Scheibel 1962 Scheibel and Scheibel 1964 1971) has been regarded to be of importance for bringing about this change.

The data described indicate that the somatosensory afferent pathways and their cortical projections have reached a comparatively high degree of structural and functional maturation in the neonate of the aforementioned animals. Consequently it is of interest to analyse the initial phases of functional development in the somatosensory system during the early prenatal period.

The classical investigations by Barcroft and Barron (1939 a,b 1941 1942) on externalized sheep fetuses kept in umbilical contact with the ewe, indicates that this preparation would be appropriate for the electrophysiological exploration of the immature central nervous system This technique was adopted by Bernhard, Kaiser and Kolmodin (1959) and several investigations from this laboratory have shown that this preparation is suitable for the electrophysiological analysis of the development of spinal and cortical functions from very early stages of ontogeny (Bergström, Bernhard and Änggård 1960 Änggård, Bergström and Bernhard 1961 Bernhard, Kaiser and Kolmodin 1962 Änggård and Ottoson 1963 Eidelberg Kolmodin and Meyerson 1965 1967 Kolmodin and Meyerson 1966 Bernhard Kolmodin and Meyerson 1967 Molliver 1967 Bernhard and Meyerson 1968 1973 Meyerson 1968 a,b Meyerson and Persson 1969 1973 Persson 1971 1973 Bernhard Meyerson and Persson 1972 Persson and Stenberg 1972) Since the sheep is relatively mature at birth from a neurophysiological and behavioral point of view (*cf* Ruckebusch 1971) the entire cycle of development of various nervous functions can be conveniently studied under the same physiological and technical conditions before a transition takes place from intrauterine to extrauterine life. In addition, basic data on the neuroanatomical development of the isocortex in fetal lambs has been presented by Åström (1967)

It has been demonstrated that the somesthetic evoked cortical response in fetal sheep undergoes a series of changes as a function of age (Molliver 1967) The characteristic surface positive wave form of the somesthetic evoked potential in immature fetal sheep (see also Meyerson and Persson 1969 Persson 1971 1973) compared to the predominately negative response of neonatal animals (*e g* Scherrer and Oeconomos 1954 Marty 1962 Thairu 1971) indicates that a different mode of cortical activation exists in the

sensory cortex during the early prenatal stages of development. The knowledge about the cortical generative mechanisms to this immature cortical activity and its structural correlates is sparse.

The present investigation on the development of somatosensory cortical functions during early prenatal ontogeny deals mainly with the following aspects

1 The functional development in the somatosensory cortex and its morphological correlates.
2 The development of the specific and nonspecific somatosensory systems.
3 The mode of operation in the immature somatosensory system.

These aspects have been studied by means of analysis of gross and unit cortical responses evoked by tactile trigeminal stimulation. The changing characteristics of these responses have been correlated to simultaneous alterations of the cortical morphology

Preliminary reports derived from this study have been presented (Meyerson and Persson 1969 Persson 1971 1973)

METHODS

I. Electrophysiology

1 Material

The results are based on 67 experiments performed on sheep fetuses of different Swedish breeds (Gotland breed and Swedish landrace) The gestational age of the experimental animals was known in most cases and in the remainder estimated from weight-age diagrams (Meyerson 1968 b) Fetal ages ranged between 42 days and full term, *i.e.* about 145 days.

2 Animal preparation

The details of the animal preparation have previously been reported (Meyerson 1968 b) and only the general outline of preparation and the control of animal condition will be presented.

On the morning of the experimental day the pregnant ewe was fastened to an operation table and anesthetized with a short acting barbiturate Thiogenal® (Merck Darmstadt) or Pentothalsodium® (Abbott) 25—30 mg/kg slowly injected into a cannulated foreleg vein. The general anesthesia was maintained throughout the surgical procedure with repeated injections (5—10 mg/kg) The need for additional anesthesia was judged from the corneal reflex.

The animal was tracheotomized and artificially ventilated with a respirator (Harvard respirator Model 613) In most of the experiments the CO_2-content of the expired air was measured (Beckman gas analyzer Model LB-1) through a small tube inserted into the tracheal cannula and continuously monitored on a Grass-polygraph The respirator was adjusted to maintain a level of 4—4.5% CO_2 in the end-tidal volume. Respiratory minute volumes were 5 0 to 10 0 l depending on the weight of the ewes (*cf* Cross, Dawes and Mott 1959)

A catheter was inserted into the proximal end of one of the ligated common carotid arteries for continuous recording of the arterial blood pressure and the pulse rate The systolic and diastolic pressure was generally about 120 and 90 mm Hg respectively and the pulse rate varied between 60 and 170 per min (*cf* Spector 1956) Care was taken to maintain the temperature of the ewe at its normal value of 39 C (*cf* Clawson 1928)

In order to avoid bronchospasm and pulmonary atelectasis an injection of adrenaline (0.2—0.4 mg s.c.) was given the animal was hoisted to an upright position a ventriculotomy was performed and the stomach contents evacuated.

The ewe was immobilized with Flaxedil® (Gallamine triethiodide May and Baker) 2—3 mg/kg i.v and decerebrated by a section placed at the level of the posterior thalamus. This operation completed the surgical procedure of the ewe and the general anesthesia was now discontinued. Throughout the experiment small doses of Flaxedil® were administered. In the dog, it has been shown that gallamine is present in fetal blood after the injection of large doses (20—50 mg/kg) into the maternal uterine artery (Pittinger and Morris 1955) However with the small doses used in the present study there was no sign of any placental transfer of the drug to the fetus.

After about two hours of recovery the fetus was carefully delivered through a caesarian section. The fetus was placed on a plastic cushion and embedded in thin cotton sheets soaked in warm mineral oil. Great care was taken not to damage the cotyledons or to stretch the umbilical cord. The body temperature *of the fetus was continuously monitored with a rectal and a skin termistor* The normal temperature (39 C) of the fetus was maintained with the aid of a heating lamp above the fetus and warm water running in the plastic cushion. Two silver pins were placed in the forelegs and connected to a Grass-polygraph for continuous recording of the fetal EKG The pulse rate of the younger fetuses (60—80 days) was 160—200/min but was lower in the fetuses near term (*cf* Barcroft and Barron 1945)

The head of the fetus was then secured to a specially designed headholder Under a dissecting microscope (Zeiss Epitechnoscope) a small or wide, unilateral or bilateral craniotomy was made according to the purpose of the experiment The dura was reflected. The exposed cortical surface was covered with thin polyethene sheets and frequently flushed with warm mineral oil.

The majority of the fetuses exhibited spontaneous mobility (*cf* Barcroft and Barron 1939 a,b 1942) and where therefore immobilized with a small dose of Flaxedil® 1—3 mg/kg intraperitoneally It has been demonstrated in the adult cat, that gallamine has excitatory effects on cuneate neurons (Galindo Krnjević and Schwartz 1968) on thalamic relay nuclei neurons (Andersen and Curtis 1964) and on cortical excitability (Halpern and Black 1967) These effects were attained with much larger doses (> 6 mg/kg) than those used in the present investigation. No significant effect on the experimental results from the use of gallamine could be observed.

3 Stimulation

As in the adult animal (Nougier 1965) ipsilateral upper lip stimulation in sheep fetuses elicited cortical responses with the most constant form, shortest latency and largest amplitude and was therefore used in the present study

Tactile stimulation was performed with a spherical probe, which had a diameter of 0.5 mm, connected to an electromagnetical transducer driven by a stimulator (Grass) One or two tactile stimulators were used each mounted on specially constructed coordinate systems allowing movements in all directions. The stimulation profile was a square wave of variable duration and amplitude In most experiments the duration of the stimulus was 10 msec. The amplitude of the stimulation was chosen so as to provide reproducible cortical responses.

Sheep fetuses have glabrous nose skin until a gestational age of about 90 days when the first hairs appear on the nose. Not until shortly before full term do the fetuses have well-developed fur Due to this fact the tactile stimulation was on bare skin in most experiments but in some older fetuses also on skin hairs.

4 Recording

Recordings were made of evoked gross potentials from the surface and the depth of the cortex as well as of evoked unitary activity from single neurons.

The surface exploring, reference and ground electrodes were all matched calomel half-cells connected to the preparation by agar saline bridges in polyethylene tubes with a recording area of 0.5 mm^2 The reference electrode was placed on saline moistened cotton in direct contact with the posterior part of the skull. The ground electrode was placed on a strip of saline moistened cotton wrapped around the neck.

The electrodes were connected to a Grass P 6 amplifier and a Tektronix 502 CRO D c. recording with an upper cut-off frequency of 0.5 or ? kHz was used in most experiments. The drift of this system was generally less than 50 μV per hour In some experiments a.c. recordings were used with a time-constant of 100 msec. A Grass kymograph camera provided the photographic recording

Evoked field potentials and extracellular single unit activity were recorded with glass microelectrodes filled with sodium chloride (5.5 N) or potassium chloride (2.8 N) It is obvious that leakage of KCl from the micropipettes could affect the single unit activity However no change could be detected in the firing pattern of spontaneously active neurons, which were extracellularly recorded with KCl microelectrodes during 15—20 minutes. Micro-

(or both) of the surface response. Furthermore on the basis of the depth potential profiles estimates were made of the corresponding vertical current gradients to achieve additional data on the location of sources and sinks'

II. Histology

After completion of the experiment, specimens were taken for histology from the somesthetic cortex of 11 fetuses (62 to 127 days of gestation) The tissue samples were fixed in formalin (10% in Ringer's solution) and stained with cresyl violet. In 8 brains the shrinkage caused by the preparation was estimated (see pp 40)

RESULTS

I. Development of somatosensory evoked surface responses

Cortical responses to tactile stimulation were obtained in all 67 fetuses examined. During development the cortical evoked response to ipsilateral upper lip stimulation underwent a series of characteristic changes in cortical distribution, configuration, latency amplitude and ability to follow repetitive stimulation. Basic data on the maturation of the somesthetic evoked surface cortical response in fetal sheep have been given in an initial study from this laboratory by Molliver (1967) The present investigation constitutes a con firmation and extension of this work mainly in order to provide a firm basis for the understanding of the depth potential data

1 Cortical distribution

The distribution on the cortex of the evoked response to tactile stimulation of the ipsilateral superior lip (ISL) was studied in fetuses of various ages. It was observed that a change of the stimulus locus from one point to another on the nose induced a shift in the cortical area from which the maximal responses could be obtained This indication of a somatotopic organization was found even in the youngest age group Thus, in a 69-day-old fetus the response could be recorded from an anterior and lateral area which included approximately the whole anterior third of the lissencephalic brain (Fig 1 A) It is interesting to note this relatively large response area and the similarity between this ISL-representation and that of the adult sheep (Adrian 1943 Hatton and Rubel 1967)

The finding in all fetuses that the responses obtained from the periphery of the cortical receiving area had the same onset latency as those in the center of the field indicates that the fringe responses do not represent electrocortical activity due to a time-consuming tangential intracortical activation

In Fig 1 B is shown the response distribution in a 76-day-old fetus and it can be seen that the cortical response was recorded from a large area of the anterior pole of the brain. A shallow impression on the lateral aspect of the brain denoted the apperance of *s suprasylvius* (*cf* Meyerson 1968 b) No responses were obtained from recordings medial to this sulcus, i.e. from the anlage of *g sup sylvius* (association cortex) nor from the prospective *g frontalis superior* (motor cortex)

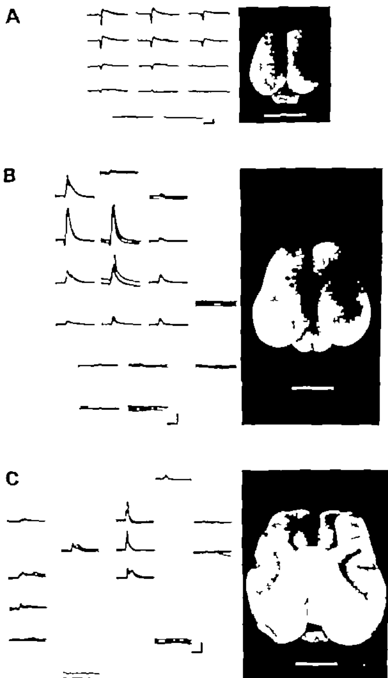


Fig. 1. Distribution of cortical responses evoked by tactile stimulation of the ipsilateral superior lip. A, 69-day fetus; B, 76-day fetus; C, 91-day fetus. The records of the cortical responses correspond to the relative positions of dots on the brains. Horizontal bars below the brains represent 1 cm. Calibration: 200 µV, 100 ms. Note: In this and subsequent figures negativity is upwards.

After the age of about 85 days, it is possible to trace the adult pattern of sulci and convolutions in fetal brains (Meyerson 1968 b) The somesthetic evoked response in a 91-day-old fetus could be recorded from *g frontalis medialis* and *g ectosylvius anterior* (Fig 1 C) These two areas presumably correspond to SI and SII respectively in the adult animal (Woolsey and Fairman 1946) No responses were obtained from *g suprasylvius* to tactile stimulation of the nose.

2 Configuration

The change in the configuration of the somatosensory evoked cortical response is a prominent developmental feature. Fig 2 shows superimposed cortical potentials (n = 3—16) evoked by tactile stimulation of the ipsilateral upper lip in fetuses of various ages. A well-defined response was obtained even in

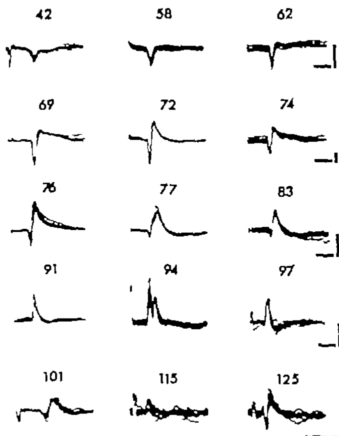


Fig. 2. Development of the configuration of evoked cortical responses in fetuses of various ages. The numbers above each record represent fetal ages in days. Calibration: 200 µV 100 ms.

the youngest fetus examined (42 days 12 g Fig 2, 42) In this as in the other fetuses younger than 68 days (Fig 2 58 and 62) the response had a surface positive form. This unique observation that the somesthetic response of sheep at its appearance in early ontogeny displays a surface positivity was first described by Molliver (1967) At a fetal age of about 68—70 days a small negativity appeared in the response after the positive deflection (Fig. 2 69) During the following developmental stage between about 70 and 80 days (Fig 2, 72 74 76 77 and 83) this negativity increased in amplitude and successively became the predominating component. During the same period the initial positivity showed a decreasing amplitude. In most fetuses of 90—100 days, the evoked cortical potential was characterized by a single surface negativity (Fig 2 91) sometimes followed by a small positive wave (Fig 2 97) However in a few fetuses responses consisting of a double negative peak were encountered (Fig 2 94) Predominating negative evoked responses to peripheral stimulation have also been recorded from the somesthetic cortex of cats (Scherrer and Oeconomos 1954 Marty 1962) rabbits (Marty 1962 Delhaye Bouchaud 1964 Verley and Rokyta 1972) and rats (Thairu 1971) during the immediate postnatal period of development. Around 100 days of fetal life in the sheep a small positive component, preceding the negativity reappeared in the response (Fig 2 101) This positivity increased in relative amplitude with age (Fig 2, 115 and 125) and at about 125 days the somatosensory cortical response displayed a positive-negative configuration similar to that of the adult animal (compare the α-response of Nougier 1963)

3 Latency

The development of the onset latency of the evoked cortical potential and the peak latencies of its main components is shown in Fig 3 A—D The onset latency of the initial positive component amounted to 115 msec in the youngest fetus (42 days Fig 3 A) This onset latency value should be compared to 0—25 msec obtained in the fetuses near term. The corresponding values of the peak latencies of the positive component were 150 msec and 30 msec respectively (Fig 3 B) The peak latency of the negative component in 69-day-old fetuses amounted to about 160 msec (Fig 3 C) The same value in fetuses older than 120 days were 40—50 msec. The latencies of the different components of the cortical response were, thus about 4—5 times longer in the younger fetuses as compared with the corresponding values in the fetuses near term (Fig 3 D) For comparison, the onset and peak latency of the positivity and negativity in adult sheep on electrical stimulation of the ipsilateral superior lip have been reported to amount to 5 12 and 17 msec respectively (Nougier 1963)

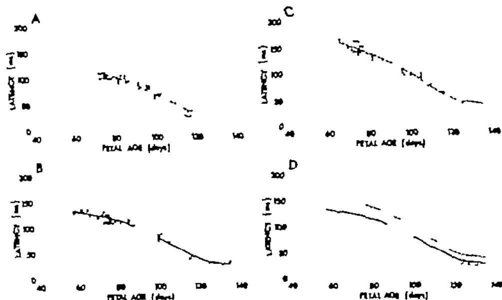


Fig. 3. Changes with age of onset and peak latencies of the evoked cortical response. A, onset latency (dots, dashed-and-dotted curve). B, peak latency of the positivity (open squares, solid curve); C, peak latency of the negativity (triangles, dashed curve). D the developmental curves of onset and peak latencies shown together (symbols as in A—C). Curves are fourth order polynomial approximations to the experimental data.

The graph in Fig. 3 A (see also Fig. 3 D) illustrating the development of the onset latencies of the somesthetic response, shows a plateau at about 110 msec between 40 and 80 days of age. There is a marked decrease of latency between the 80th and the 120th day of gestation after which the latency levels off. The peak latencies showed a continuous decrease to about the 120th day and then a levelling off (Fig. 3 B, C; see also Fig. 3 D).

4. Amplitude

When the intensity of the tactile stimulation was increased stepwise above the threshold, the peak amplitude of the cortical response increased at each step and finally reached a level of saturation. The range in response amplitude from the threshold to the saturation level had a tendency to be larger in younger than in older fetuses. The data plotted in Fig. 4 illustrating the development of the peak to-peak amplitude of the evoked potential were derived from responses obtained with supramaximal stimulation. The maximal responses in the fetuses younger than about 63 days were of low amplitudes (150—600 μV). Between 65 and 76 days, cortical responses could be obtained with peak to-peak amplitudes of up to 1 500 μV. However, there was

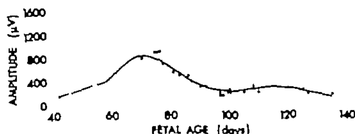


Fig. 4 Development of the peak-to-peak amplitude of the evoked cortical response. Curve is eighth order polynomial approximation to the experimental data.

a large variability in the amplitude of the responses recorded from fetuses of that age and low-amplitude evoked potentials were also observed. During the following developmental period the cortical responses showed a progressive diminution and from a fetal age of about 100 days the potentials had an amplitude of 200—400 μV Such low amplitude values are typical for the adult animal (for sheep see Nougier 1963)

5 Repetitive stimulation

In several investigations it has been shown that the ability of evoked cortical responses to follow repetitive peripheral (Hunt and Goldring 1951 Scherrer and Oeconomos 1954 Grossman 1955 Molliver 1967 Myslivecek 1968 a, Thairu 1971 Briquel and Verley 197? Persson and Stenberg 1972) and central stimulation (Purpura 1961 a,b Scheibel and Scheibel 1964 1971 Grafstein 1963 Meyerson 1968 a,b, Conway Wright and Bradley 1969) increases significantly during development. In sheep fetuses younger than 70 days of age the tactile stimuli had to be given at intervals of 20—30 seconds to obtain reproducible cortical responses. In 75-day-old fetuses, the evoked cortical potential could follow a stimulus frequency of about 0.5 Hz, whereas the response followed a rate of about 1 Hz in fetuses older than 88 days. For comparison it may be mentioned that in nonanesthetized adult cats the recovery time of the primary somatosensory response has been reported to last ?00 msec corresponding to a repetition rate of 5 Hz (Allison 1968)

In order to evaluate the susceptibility of the various response components to repetitive stimulation, experiments were performed in which the rate of stimulation was increased above that giving responses of constant amplitude. Fig 5 A and B shows a series of successive cortical potentials obtained at a stimulus rate of 1 Hz in a 65- and a 7?-day-old fetus respectively In the 65-day-old fetus the responses were reduced to a low-amplitude positive deflection followed by a slow negative wave (Fig 5 A) The corresponding

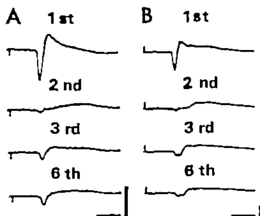


Fig. 5. Cortical responses to repetitive stimulation (1 Hz) in a 65-day fetus (A) and a 72-day fetus (B) Calibration ?00 µV 100 ms.

data (Fig 5 B) from the 72-day-old fetus show that the change in the cortical response was similar between the first and the second stimulation. However in the third response additional components appeared as represent ed by the second positive deflection and the negative hump superimposed on the slow negative wave (see also Fig 19)

Facilitation was observed in a few fetuses between 80—120 days of age. In Fig 6 is shown an example of such a facilitation in the response of an 82-day-old fetus induced by repetitive stimulation at a rate of 1 Hz. From the second stimulation there was a remarkable increase in amplitude of both the positive and the negative components. The facilitation appeared in a waxing and waning fashion and the negativity was split up into two peaks. Facilitation was never observed in younger fetuses. The alterations in the cortical response in older fetuses during high frequency stimulation were highly inconsistent.



Fig. 6 Facilitation of the cortical response in n 82-day fetus by repetitiv stimulation of 1 Hz Calibration 100 µV 200 ms.

6 Effect of strychnine

In an attempt to elucidate the development of inhibitory mechanisms in the cortex some experiments were undertaken in which the effect of strychnine on the evoked cortical potential was studied. In Fig 7 A is reproduced evoked responses in a 76-day-old fetus before and after topical application of 1% strychnine on the cortex. After strychnine there was a slight prolongation of the negative component. No significant change occurred in the response amplitude. Fig 7 B illustrates corresponding data from a 91 day-old fetus. After strychnine prominent changes could be observed in the response which displayed a twofold increase in the peak to-peak amplitude together with a marked prolongation of the duration. In addition a small initial positivity appeared and there was also a hump on the rising phase of the negativity On the assumption that strychnine blocks inhibitory post synaptic mechanisms in the cortex (Pollen and Ajmone Marsan 1965 Stefanis and Jasper 1965 Phillis and York 1967) the fact that strychnine only had an effect on the somesthetic response in older fetuses may be taken as evidence of a delayed maturation of cortical inhibitory synaptic systems during early periods of ontogeny

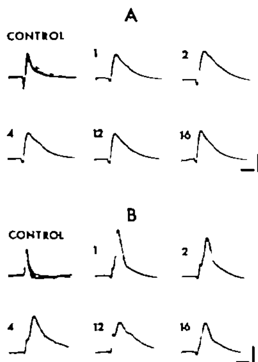


Fig 7 Effect of topical strychnine (1 %) on the evoked cortical response in 76-day fetus (A) and 91-day fetus (B) Numbers above each record represent time in min after strychnine application Calibration 200 µV 100 ms.

7 Afterdischarges

In 6 older fetuses (aged 85—120 days) it was possible to evoke cortical afterdischarges in addition to the primary evoked potential (Fig 8 A—C) The afterdischarge usually consisted of an epoch of spindling activity superimposed on a negative d.c.-shift The onset latency of the discharge amounted to 400—600 msec and it had a duration of 400—800 msec. The frequency of the waves in the afterdischarge varied from 8—16 Hz. The negative d.c.-shift was generally more pronounced in the older fetuses in which it could attain a value of about 400—600 μV

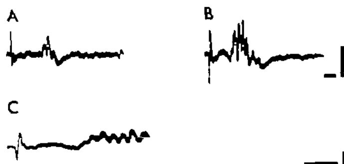


Fig 8. Cortical afterdischarges evoked by tactile stimulation f the trigeminal nose region. A, a 94-day fetus B, 100-day f tus C, a 105-day fetus Calibration 200 μV 200 ms.



Fig 9 Selective effect of repetitive stimulation (0.5 Hz) on the 'primary' evoked response and the afterdischarge in 100-day fetus. Calibration: 100 μV 100 ms.

The cortical afterdischarge had a comparatively low resistance to repetitive stimulation even compared to that of the primary evoked response As shown in Fig 9 the afterdischarge was almost completely abolished at a stimulation rate of 0.5 Hz, whereas the primary response was relatively unaffected. This finding may indicate that the two responses are generated by different subcortico-cortical mechanisms.

II. Development of somatosensory evoked field potentials

In order to evaluate the relative contribution of the activity in the different cortical layers to the building up of the evoked surface response during development, a series of experiments was performed in which the evoked potentials were recorded at different cortical depths. Because the development of the evoked surface response is characterized by a series of successive changes in the size of its positive and negative components, laminar potential analyses were based on the amplitudes of the evoked field potentials at the latencies corresponding to the peaks of the surface positivity and negativity. On the basis of the measured potential values, depth-potential profiles and estimates of the corresponding vertical current gradient were made (see Methods). The results to be presented comprise laminar analysis of the somatosensory evoked cortical potentials from five successive developmental stages each characterized by a specific pattern of the surface response.

62-day-old fetus

The results of the laminar potential analysis in a 62-day-old fetus exhibiting a surface positive cortical response are shown in Fig. 10. Upper traces in A represent the surface potential and lower traces illustrate corresponding field potentials recorded with a microelectrode at indicated depths. In recordings from depths less than 1 000 μ the field potentials displayed a monopolar positive form, similar to the surface response. At greater depths the field potentials exhibited negative values with maximal amplitudes at a depth of about 2 000 μ. The peak latency of the deep negative wave was 115 msec which corresponded to that of the surface positivity. The cortical thickness amounted to 700 μ (horizontal dotted line in Fig. 10 B and C, see pp. 40). Thus the positivity of the surface response corresponds to positive field potentials located within the primitive cortex and to negativities located in the upper subcortical strata. The depth potential profile shows that the maximal negative values of the field potentials—corresponding to the peak of the surface positivity—are located in subcortical layers (Fig. 10 B).

The graph in Fig. 10 C illustrates the vertical current gradient derived from the depth potential profile. On the assumption that the negative maximum represents a net maximal current sink, this is located at a depth of about 1 200 μ. Similarly, if the positive maximum represents a net maximal source, this is situated at about 1 000 μ. To check the location of the microelectrode tips, marking experiments were performed with the Prussian blue method. The result revealed that the deep negativity and the net current sink were located in the subcortical strata (intermediate layer) of the immature telencephalic wall.

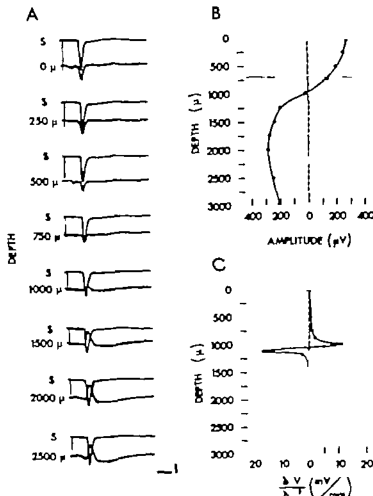


Fig. 10. Laminar analysis of evoked cortical potentials in a 62-day fetus. The upper records in A are surface potentials lower re field potentials recorded with a microelectrode t indicated depths. Calibration 200 μV 100 ms. Depth-potential profile (B) and estimate of the vertical current gradient (C) corresponding to the peak of the surface positivity A positive value of the gradient curve indicates a source of vertical current and negative value indicates a relative sink (see Methods) The horizontal dotted lines (B and C) represent the approximate cortical thickness.

These observations indicate that early in development tactile stimulation of the trigeminal nose region does not activate neuronal elements within the cortex proper but evokes activity in strata immediate below the cortex.

70-day-old fetus

Laminar analysis of somatosensory evoked cortical potentials in a 70-day-old fetus is illustrated in Fig 11 At this stage the surface response displayed

gradient graph were located at superficial (350 μ) and deep cortical strata (1 000 μ Fig 13 C) In between there was a high amplitude positivity in the vertical current gradient curve which may represent a midcortical net current source The results obtained may indicate the existence of two preferential loci of neuronal activity corresponding to the cortical layers I—II and IV—V (see Fig 25 D)

110-day-old fetus

Fig 14 A shows the evoked cortical potentials from a 110-day-old fetus displaying a positive-negative surface response The records of the field-potential show that during penetration the initial positive component dwindled quickly while the negative component grew and remained of high amplitude throughout the cortex. A shortening of the peak latency of the negative component was observed in superficial layers, and at a depth of 1 000 μ it had a latency amounting to 70 msec, which corresponded to the peak of the surface positivity The net maximal current sink (Fig 14 B, right) corresponding to the surface positivity was located in the midcortex at the approximate depth of the layer IV (see Fig 25 E) There was indication of two net current sources above and below this sink. The corresponding net maximal current sink to the surface negative wave was located in the uppermost part of the cortex (Fig 14 C right)

III Development of somatosensory evoked single unit activity

1 Response characteristics

Altogether 228 single unit responses were recorded in 3? sheep fetuses aged between 65 and 125 days. Samples of evoked single unit responses are shown in Fig 15—18 (lower records) In the fetuses younger than 68 days of age the unitary responses (9 of 12 units) generally consisted of a repetitive discharge displaying 2—4 spikes (Fig 15) The evoked unit activity recorded from the cortex of fetuses between 70 and 90 days was always a single discharge (Fig 16) Neither an increase of the intensity of the tactile stimulation nor an alteration of the stimulus locus on the nose caused a recruitment of additional spikes, contrary to what is found in the adult animal (Mountcastle 1957 Mountcastle, Davies and Berman 1957 Towe and Kennedy 1961)

Between 90—110 days of fetal age single spike responses were encountered from the majority of the neurons (Fig 17 96, left and 105) Occasionally repetitive discharges were observed (Fig 17 96 right and 103) At the same developmental stage a few neurons were seen to fire concomitantly with the cortical surface afterdischarge (Fig 24) During subsequent deve-

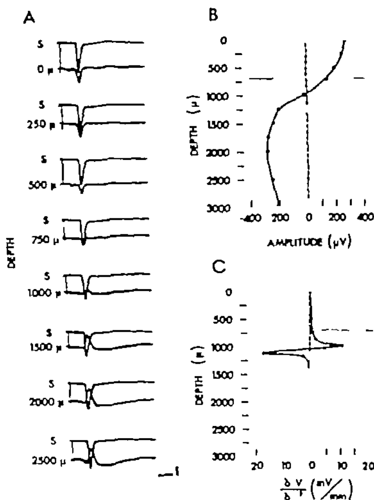


Fig. 10. Laminar analysis of evoked cortical potentials in a 62-day fetus. The upper records in A are surface potentials; lower are field potentials recorded with a microelectrode at indicated depths. Calibration 200 μV 100 ms. Depth-potential profile (B) and estimate of the vertical current gradient (C) corresponding to the peak of the surface positivity. A positive value of the gradient curve indicates source of vertical current and negative value indicates a relative 'sink' (see Methods). The horizontal dotted lines (B and C) represent the approximate cortical thickness.

These observations indicate that early in development tactile stimulation of the trigeminal nose region does not activate neuronal elements within the cortex proper but evokes activity in strata immediate below the cortex.

70-day-old fetus

Laminar analysis of somatosensory evoked cortical potentials in a 70-day-old fetus is illustrated in Fig 11. At this stage the surface response displayed

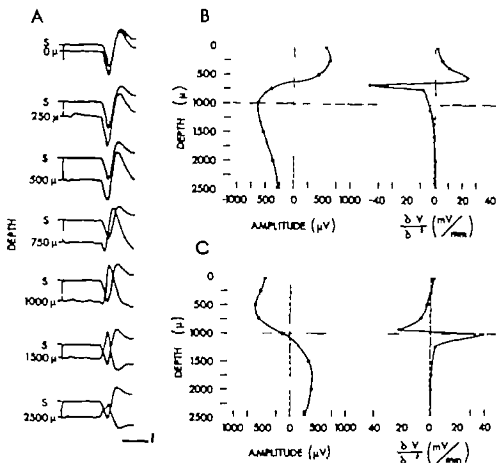


Fig. 11 Laminar analysis of evoked cortical potentials in a 70-day fetus. The upper records in A are surface potentials lower are field potentials recorded at indicated depths. Calibration 200 μV 100 ms. B, depth-potential profile (left) and estimate of the vertical current gradient (right) at the peak of the surface positivity C, the corresponding data at the peak of the surface negativity The horizontal dotted lines (B and C) represent the approximate cortical thickness.

a biphasic positive negative form with a predominance of the positive component (Fig 11 A, upper traces) The configuration of the evoked field potentials (Fig 11 A, lower traces) recorded at depths down to 250 μ was almost identical to the surface response At increasing depths the field potentials displayed a gradual shift in wave form which could be characterized as a gradual shortening in the latency of the negative wave which eventually led to masking of the positivity at the depth of 1 000– 1 500 μ A maximal amplitude of the negative potential was met at a depth of 1 000 μ The shortest onset latency of the deep negativity was observed in responses obtained at depths of 1 500—2 500 μ and it appears from the records that at

these depths the field potentials have a monophasic negative configuration In this developmental stage the lower border of the cortex was found to be situated at a depth of about 1 000 μ (horizontal dotted line in Fig 11 B and C, see pp 40)

The graphs in Fig. 11 B show the depth potential profile (left) and the vertical current gradient (right) corresponding to the peak of the surface positivity As seen the net maximal current sink was confined to the deep layers of the cortex. Negative values of the vertical current gradient curve correspond to depths of the primordia to cortical layers IV—VI (see Fig 25 B) Positive values of the gradient were obtained in superficial cortical layers. The depth-potential profile (Fig 11 C, left) and vertical current gradient (Fig 11 C, right) of the surface negativity reveal a predominantly deep intracortical location of the net current sink with its maximum at 950 μ The net current source was located at the lower border of the cortex proper

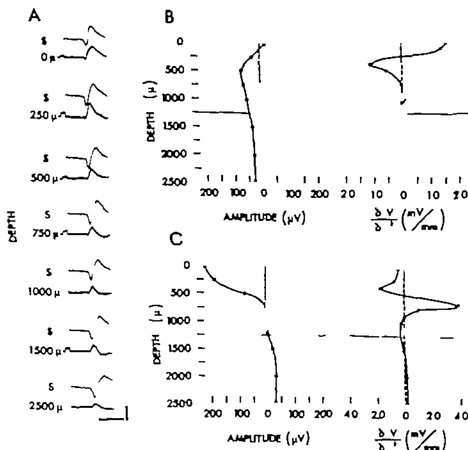


Fig. 12 Laminar analysis of ev ked cortical potentials in an 80-day f tus. For f rther description see F g. 11

These findings indicate that from 68—70 days of fetal life the neurons *within* the deeper layers of the cortex can be activated by the trigeminal afferent inflow

80-day-old fetus

The evoked field potentials in the cortex of an 80-day-old fetus displaying a surface positive-negative response with a predominating negativity show that with increasing depth the biphasic response was transformed into a monophasic negativity (Fig 12 A) This change in configuration was similar to that observed in the previous stage although it now took place in more superficial strata (down to 250 μ, Fig 12 A, lower traces) With increasing depths down to 2 500 μ the evoked field potentials remained negative. The depth-potential profile (Fig 12 B left) and estimate of the vertical current gradient (Fig 12 B right) of the peak of the surface positivity show negative

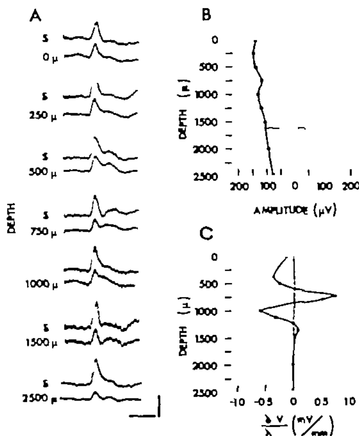


Fig. 13 Laminar analysis of evoked cortical potentials in 94-day fetus. Depth-potential profile (B) and estimate of the vertical current gradient (C) corresponding to the peak of the surface negativity For f rther description, see Fig. 10

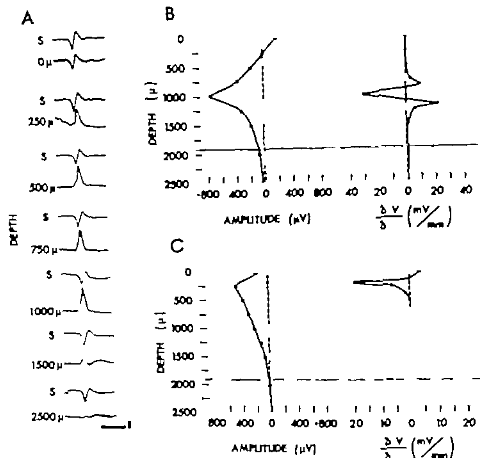


Fig. 14 Laminar analysis of evoked cortical potentials in 110-day fetus. For further description, see Fig. 11

maxima in the superficial cortical strata which later form the layers II—III (see Fig 25 C) The corresponding data for the negative wave (Fig 12 C) reveal net current sinks in the prospective layers I—III and in the deep strata of the cortex. The positive value of the vertical current gradient may correspond to a midcortical net current source. These results with prominent superficial current sinks are consistent with the assumption that the sensory stimulation now activated neurons throughout the cortex and that there is a predominance of activity in superficial strata.

94-day-old fetus

During the developmental period in which the evoked response had a monopolar negative form, the field potentials remained negative at all depth recordings (Fig 13 A and B) Two negative peaks in the vertical current

gradient graph were located at superficial (350 μ) and deep cortical strata (1 000 μ Fig 13 C) In between, there was a high amplitude positivity in the vertical current gradient curve which may represent a midcortical net current source. The results obtained may indicate the existence of two preferential loci of neuronal activity corresponding to the cortical layers I—II and IV—V (see Fig 25 D)

110-day-old fetus

Fig 14 A shows the evoked cortical potentials from a 110-day-old fetus displaying a positive-negative surface response The records of the field potential show that during penetration the initial positive component dwindled quickly while the negative component grew and remained of high amplitude throughout the cortex. A shortening of the peak latency of the negative component was observed in superficial layers, and at a depth of 1 000 μ it had a latency amounting to 70 msec, which corresponded to the peak of the surface positivity The net maximal current sink (Fig 14 B, right) corresponding to the surface positivity was located in the midcortex at the approximate depth of the layer IV (see Fig 25 E) There was indication of two net current sources above and below this sink. The corresponding net maximal current sink to the surface negative wave was located in the uppermost part of the cortex (Fig 14 C, right)

III. Development of somatosensory evoked single unit activity

1 Response characteristics

Altogether 228 single unit responses were recorded in 32 sheep fetuses aged between 65 and 125 days. Samples of evoked single unit responses are shown in Fig 15—18 (lower records) In the fetuses younger than 68 days of age the unitary responses (9 of 12 units) generally consisted of a repetitive discharge displaying 2—4 spikes (Fig 15) The evoked unit activity recorded from the cortex of fetuses between 70 and 90 days was always a single discharge (Fig 16) Neither an increase of the intensity of the tactile stimulation nor an alteration of the stimulus locus on the nose caused a recruitment of additional spikes contrary to what is found in the adult animal (Mountcastle 1957 Mountcastle Davies and Berman 1957 Towe and Kennedy 1961)

Between 90—110 days of fetal age single spike responses were encountered from the majority of the neurons (Fig 17 96 left and 105) Occasionally repetitive discharges were observed (Fig 17 96 right and 103) At the same developmental stage a few neurons were seen to fire concomitantly with the cortical surface afterdischarge (Fig 24) During subsequent deve-

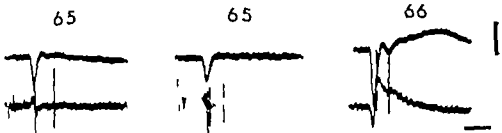


Fig. 15—18. Evoked surface and single unit responses in fetuses of various ages. The numbers above each record represent fetal ages in days. Calibration 200 μV 100 ms.

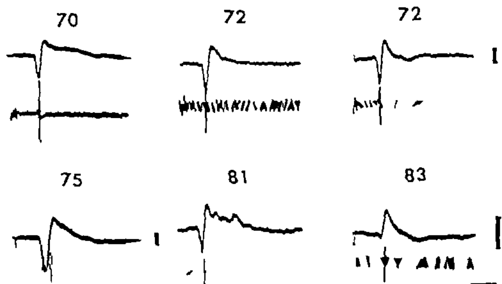


Fig 16. Legend, see Fig. 15

lopment the relative number of evoked repetitive unit discharges increased. Thus, in a 115-day-old fetus most of the cortical neurons (12 of 17 units) responded with repetitive spikes (Fig 18 right records) The train of discharge consisted of one to four spikes. Similar modal values' of cortical neurons have been observed in the somesthetic cortex of the adult cat (Mountcastle *et al.* 1957) From the age of about 100 days, it was possible to establish a relationship between the relative site of the stimulation within the excitatory receptive field of the neuron and the intensity of stimulation on one hand and the pattern of discharge on the other Thus, decreasing of the stimulation intensity or changing of the site of stimulation away from the center of the excitatory receptive field resulted in a reduction of the number of spikes per response and a lengthening of initial response latency All these response properties have been found to be characteristic for the

gradient graph were located at superficial (350 μ) and deep cortical strata (1 000 μ, Fig 13 C) In between there was a high amplitude positivity in the vertical current gradient curve which may represent a midcortical net current source The results obtained may indicate the existence of two preferential loci of neuronal activity corresponding to the cortical layers I—II and IV—V (see Fig 25 D)

110-day-old fetus

Fig 14 A shows the evoked cortical potentials from a 110-day-old fetus displaying a positive-negative surface response The records of the field-potential show that during penetration the initial positive component dwindled quickly while the negative component grew and remained of high amplitude throughout the cortex. A shortening of the peak latency of the negative component was observed in superficial layers, and at a depth of 1 000 μ it had a latency amounting to 70 msec, which corresponded to the peak of the surface positivity The net maximal current sink (Fig 14 B, right) corresponding to the surface positivity was located in the midcortex at the approximate depth of the layer IV (see Fig 25 E) There was indication of two net current sources above and below this sink. The corresponding net maximal current sink to the surface negative wave was located in the uppermost part of the cortex (Fig 14 C, right)

III. Development of somatosensory evoked single unit activity

1 Response characteristics

Altogether 228 single unit responses were recorded in 32 sheep fetuses aged between 65 and 125 days. Samples of evoked single unit responses are shown in Fig 15—18 (lower records) In the fetuses younger than 68 days of age the unitary responses (9 of 12 units) generally consisted of a repetitive discharge displaying 2—4 spikes (Fig 15) The evoked unit activity recorded from the cortex of fetuses between 70 and 90 days was always a single discharge (Fig 16) Neither an increase of the intensity of the tactile stimulation nor an alteration of the stimulus locus on the nose caused a recruitment of additional spikes, contrary to what is found in the adult animal (Mountcastle 1957 Mountcastle, Davies and Berman 1957 Towe and Kennedy 1961)

Between 90—110 days of fetal age single spike responses were encountered from the majority of the neurons (Fig 17 96 left and 105) Occasionally repetitive discharges were observed (Fig 17 96 right and 103) At the same developmental stage a few neurons were seen to fire concomitantly with the cortical surface afterdischarge (Fig. 24) During subsequent deve

frequency burst of activity (*cf* Huttenlocher 19— from the high frequency discharge found in adu... 1961)

2 Repetitive stimulation and recovery cycles

In order to get a further idea of the development ... somatosensory system to transmit closely spaced ... made in a few experiments of the effect of repetitive ... on the evoked unit activity in fetuses of various ... the evoked unit responses were generally more ... lation than the evoked surface potentials. The ... induced considerable alterations in the wave form ... tentials.

The responses depicted in Fig 19 were obtained ... stimulated with a frequency of 1 Hz Except for the ... evoked unit activity fired in response to stimulation ... At higher frequencies the unit failed to follow and ... ally It further appears that the amplitude of the ... potential decreased during repetition and that it ... towards splitting into two minute positive components ... concomitant unit activity from the third stimulus ... with the second of these positivities. An interpretation ... positive component of the positivity is of presynaptic ... and the negativity are of postsynaptic origin (*cf* Fig

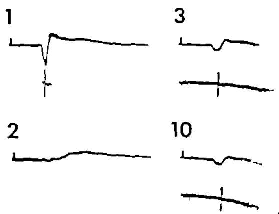


Fig. 19 Effect of repetitive stimulation (1 Hz) on ... sponses in 72-day fetus. Calibration 200 µV 200 ...



Fig 20. Effect of paired stimulation on evoked surface and single unit responses in an 82-day fetus (A) with stimulus intervals of 400 msec (left) and 300 msec (right) and in a 105-day fetus (B) with stimulus intervals of 200 msec (left) and 100 msec (right) Arrows, time of stimulation. Calibration 200 µV 200 msec.

examples of responses to paired stimulation in an 82-day-old fetus with a separation of the two stimuli amounting to 400 and 300 msec respectively At stimulus intervals of 400 msec or more both stimuli caused a discharge. A similar experiment is illustrated in Fig 20 B in a 105-day-old fetus and it appears that with intervals of 200 msec or more the second stimulation evoked a single unit response.

3 Relation to evoked surface responses

The time relation of single neuronal responses to the evoked surface potentials in fetuses of different ages can be observed in Fig 15—18 In fetuses younger than 68 days, the initial spike of the evoked units discharged at a time corresponding to the peak of the surface positivity In Fig 21 is illustrated the latencies of the recorded units in relation to the evoked surface responses for four developmental stages. Each of these stages is characterized by a typical configuration of the surface response. It should be noted, however that data from the initial developmental stage are not included since the number of units recorded was too small. The black histograms represent the latencies of the initial spike, whereas the dotted areas represent those of the second spike In the developmental period

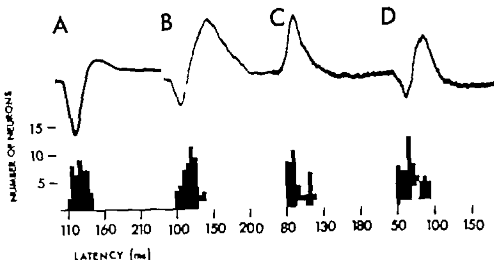


Fig. 21 Latencies of evoked single unit discharges in relation to the surface response in four developmental stages (A—D) Each of these stages is characterized by the configuration of the surface response. The black histograms represent the latencies of the initial spike, whereas the dotted areas represent those of the secondary spike. *Note* the data from the initial developmental stage are not included.

comprising fetuses of 68 to 75 days of age, all evoked single units discharged during the course of the positivity with a maximum at its peak (Fig 21 A) During the next stage (Fig 21 B) the single unit responses fired during the positivity and the initial phase of the negativity with a maximum at a latency value corresponding to the onset of the negativity In these two stages the neuronal responses always consisted of a single spike.

The evoked units concomitant with the monopolar negative response occured during the entire time course of the response with a preference of discharge during the initial phase (Fig 21 C) In fetuses older than 100 days characterized by a positive negative surface response the evoked units fired during the positivity and the initial phase of the negativity (Fig 21 D) The dotted histograms in Fig 21 C and D represent the latencies of the secondary spikes of the units with repetitive discharge found in fetuses older than 90 days.

4 Depth-distribution

In the fetuses less than 68 days of age it was not possible to record more than 12 units despite the fact that a large number of penetrations (about 100) were performed with microelectrodes of different recording characteristics. These 12 units were all encountered at depths of 1 300—2 800 μ below the cortical surface. In order to check the depths from which these units were

negative d c.-shift (*cf* Fig 8) It was possible to show that this afterdischarge may be accompanied by repetitive unitary responses (Fig 24) The left record shows a unit which discharged in conjunction with the primary' response and during the afterdischarge. Another type of discharge more frequently encountered is illustrated in the right record where there was activity only during the cortical afterdischarge No constant relation could be observed between the spikes and the individual waves of the afterdischarge.

IV *Development of cortical morphology*

Some data on the early morphological development of the neocortex in fetal sheep are presented in this section to facilitate the understanding of the relationship between structure and function. A great deal of information has been collected in an extensive investigation by Åström (1967) In the present study some complementary results will be presented with special regard to the maturation of the cortical stratification Such data are of particular significance in relating the neuronal events to the cortical structure.

In Fig 25 is depicted a series of brain sections stained with cresyl violet, from fetuses of various ages The specimens were taken from the cortical areas in which somesthetic evoked responses were recorded. In the older fetuses with gyrencephalic brains these areas correspond to the area praeparietalis of the adult animal (Rose 1942)

During the early developmental period when the fetal brain is lissencephalic, the neopallial wall is made up of three distinct concentric strata the cortical plate the intermediate zone and the germinal layer (Åström 1967) The immature cortex proper consists of two layers, the marginal and pyramidal layers, which successively develop into the 6-layered adult neocortex. In the brain section from a 62-day-old fetus (Fig 25 A) the cortex proper and the upper subzone of the intermediate layer can be seen. This type of stratification is characteristic for an early phase of neocortical ontogeny The lightly stained marginal layer (a) contains horizontally orientated cells, the Retzius-Cajal cells (Retzius 1891 1893 Cajal 1960 Åström 1967) An incipient stratification can be observed in the pyramidal layer (b—d) with an indication of a light band in its middle third (c) The cells in the pyramidal layer have an immature appearance in the outer part small dark cells of bipolar shape are arranged in compact vertical columns in the inner layers a widening of the intercellular spaces can be observed. The subpyramidal layer (e) contains some large cells with a relative mature appearance presumably stellate cells (*cf* Åström 1967)

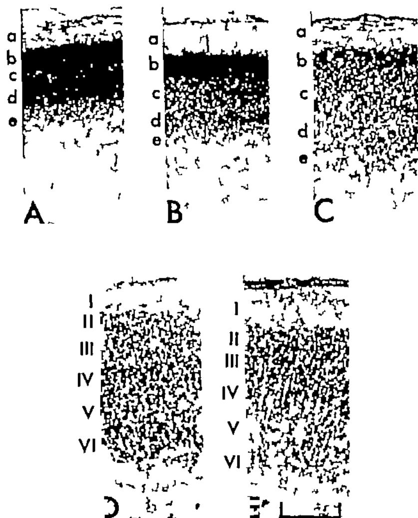


Fig. 25 Somesthetic cortex t different stages of maturation. Brain sections from f tuses of 62 (A) 69 (B) 82 (C) 98 (D) and 127 days of age (E) Cresyl violet stain. Horizontal bar represents 500 μ.

The lamination of the cortex in a 69-day-old fetus stands out more clearly and consists of 5 identifiable layers (Fig 25 B) The uppermost part (b) of the pyramidal layer is crowded with cells of the same appearance as in the younger animals. Compared to earlier stages the light band in the middle of the pyramidal layer (c) has become wider and more prominent. This is probably due to the fact that the evolvement of dendritic and fibrillar processes has caused a separation of cells and consequently a widening of the intercellular spaces (Lorente de Nó 1933 Åström 1967) The pyramids

of the deep dark layer (d) are less packed and more mature than those in the superficial pyramidal layers (b) Large stellate cells can be observed for the first time within the inferior pyramidal layer These cells are the largest neurons in the neocortex at this developmental stage a fact which may be of functional significance in the present context. The cellular morphology in this stage has been described by Åström (1967) in detail with the aid of the Golgi method.

The section (Fig 25 C) from a 82-day-old fetus represents the next developmental stage and a more definite 5-layered stratification is now discernable. In the Nissl preparations most cells appear to have reached a comparatively advanced stage of development. However the most superficial cells are still immature and form a thin dark stratum (b) below the marginal layer (a)

A clear 6-layered stratification similar to what is found in the adult animal (Rose 1942) is observed in the cortex of a 98- and a 127-day-old fetus (Fig 25 D and E) Mature pyramidal cells are found in all cortical layers even in the most superficial ones.

To summarize the cortical cells and strata, as studied in Nissl stained preparations become differentiated in a definite sequence. The marginal and the subpyramidal layers are the first to become differentiated These strata will form the layer I and the lower part of layer VI in the adult cortex. Thereafter the lightly stained stratum of the pyramidal layer develops and will become the layers IV and lower III Parallel with these changes, stellate cells are incorporated in the lower pyramidal layers. The deep pyramids which constitute layers V and VI will then attain their mature appearance During the subsequent development the cells within layers III and II differentiate to their mature form (*cf* Åström 1967)

For the interpretation of the results in the current investigation the reliability of the estimations of the depth locations of neuronal activity as well as its morphological substrate are of paramount importance Therefore it is necessary to know the degree of shrinkage induced by the histological preparation For this purpose an iron deposit was produced through a microelectrode tip at a fixed cortical depth as read off by the micrometer during an actual experiment (*e.g.* Änggård 1965) By comparing the depths of the Prussian blue spots in brain sections with the corresponding micrometer readings the shrinkage factor could be determined. In the present fetal material the shrinkage of the neocortex amounted to about 30% (range 25—35%) which is somewhat larger than the corresponding value (20—25%) reported for the adult rat cortex (Borbély 1970) This difference may be explained by the fact that the water content of the immature brain is higher

than that of the mature brain (*e.g.* Vernandakis and Woodbury 1962) In Fig 26 A is shown an example of an iron deposit produced at a depth of 500 μ in the neocortex of a 71-day-old fetus contrastained with cresyl violet. This deposit was located at the border between the immature superficial dark (prospective layers II and III a *cf* Fig 25 B layer b) and the relatively more mature middle light layers (prospective layers III b and IV *cf* Fig 25 B, layer c)

The markings shown in Fig 26 B are from an 82-day-old fetus. The whole track down to 500 μ was marked and the lower limit corresponded to the superficial cortical strata (layers II and III *cf* Fig 25 C layer b) The deposit at 1 000 μ was located at the lower border of the prospective layer IV (*cf* Fig 25 C, layer c) On the basis of the shrinkage factor of 30% the approximate cortical thickness was determined from the cresyl violet stained brain sections made at different developmental stages 700 μ at the age of 62 1 000 μ at 72 1 250 μ at 82 1 650 μ at 98 and 1 900 μ at 127 days. These values have been used to denote the cortical thickness in Fig 10 11 12 13 14 and 22



Fig. 26 Prussian blue markings in cortical sections stained with cresyl violet. A, t a depth of 500 μ in 71-day fetus; B t depths of 500 μ and 1 000 μ in an 82-days fetus. Horizontal bar represents 500 μ.

DISCUSSION

The present results demonstrate that a somesthetic response to tactile stimulation can be recorded from the cortical surface of sheep fetuses already at a very early stage of ontogeny thus confirming the original study by Molliver (1967) During the subsequent development the response under goes a sequence of characteristic alterations. Earlier investigations on cortical potentials evoked by somesthetic stimulation in dogs, cats, rabbits and rats during the postnatal period show that a response is present at birth (Scherrer and Oeconomos 1954 Grossman 1955 Marty 1962 Delhaye-Bouchaud 1964 Tharu 1971 Verley and Rokyta 1972) A comparison of the functional characteristics of the cortical response in these altricial postnatal animals and in the fetal sheep of different ages suggests that the developmental stage of the somatic sensory system of newborn dogs, cats, rabbits and rats corresponds approximately to that of the 80—90-day-old fetal sheep In addition, the sequence of changes in the response, in particular with regard to its configuration during the postnatal period of these animals is very similar to those observed in the later prenatal stages of sheep These observations lend support to the notion that the difference in rate of pre- and postnatal functional development of these species (Adolph 1970) also applies to the somatosensory system (*cf* Gottlieb 1971) Thus, the present study on fetal sheep covers the initial periods of development of the somatosensory system, whereas earlier investigations on postnatal animals have been confined to relatively more mature stages of ontogeny

1 Aspects of the mode of operation in the immature somatosensory system

Unitary recording in the adult cortex is subject to bias (Towe and Harding 1970) This bias may be even more important in the case of immature neurons, which are small and have fragile membranes as deduced from their structural and functional properties different from those found in the adult (*e g* Purpura 1972) As a matter of fact, stable recordings during long lasting sessions are very difficult to obtain and impossible to achieve in the early prenatal cortical stages Nevertheless, a large number of penetrations were made into the subcortex at each developmental stage with micro-

electrodes of different recording characteristics. It should be recalled that general anesthesia shown to decrease responsiveness of cortical neurons (Mountcastle *et al.* 1957) has not been used. It cannot be excluded that factors at the stimulation site *e.g.* changes in the tissue properties and size of the stimulated nose area in the relation to the stimulating probe may influence results of the present study such as the development of amplitude and cortical distribution of the response. However there are reasons to believe that these factors do not contribute significantly and in a predictable way to the parameters of development observed Thus, for instance, the response amplitude shows both an increase and a decrease during the period of development when the relative stimulation area decreases.

The functional significance of the sensory evoked repetitive discharge in the subcortical units of the young fetal sheep is unknown. A similar pattern of discharge of subcortical neurons evoked by light stimulation has been observed in the newborn kitten (Huttenlocher 1967) When, during development evoked units were first obtained within the cortex they responded with a single spike. Such a discharge pattern has also been demonstrated in experiments on newborn kittens and shown to represent postsynaptic activation (Purpura *et al.* 1965) Another aspect of the low level of responsiveness of the immature cortical neurons is their characteristic paucity of spontaneous discharge (Persson, in preparation *cf* Hyvärinen 1966 Huttenlocher 1967 Laget, Thomson and Delhaye Bouchaud 1967 Armstrong James 1970 Rubel 1971) Contrary to what has been found in the adult animal (Mountcastle 1957 Mountcastle *et al.* 1957 Towe and Kennedy 1961 Duran-Smith 1966) the immature cortical neuron responds only with a single spike regardless of the strength and location of the stimulus within the peripheral receptive field This finding points to a fundamental difference in the function of the immature and the mature somatosensory system to code information about the stimulus parameters. The appearance during development of cortical neurons capable of evoked repetitive discharge denotes the transition to a stage when the somesthetic system begins to operate in a way qualitatively similar to that of the adult. It is important to recall that this maturational process is highly dependent on extracortical functions such as an increased maturity of the tactile cutaneous receptors and of the peripheral sensory nerves (Ekholm 1967 Kasprzak, Tapper and Craig 1970)

Contrary to the findings in a 76-day-old fetus, the clear-cut releasing effect of strychnine on the evoked potential in a 90-day-old fetus as well as the presence of cortical units at a comparable developmental stage, whose spontaneous discharge was arrested by peripheral stimulation, strongly suggest that inhibitory mechanisms within the cortex are effective at that later stage

of development In corresponding developmental periods of postnatal kittens, pronounced long lasting IPSP:s have been observed in cortical neurons as a result of electrical stimulation in the thalamus (Purpura *et al* 1965) Further more, the duration of these IPSP:s is of the same order of magnitude as the duration of the inhibitory arrest of spontaneous units observed in the present study These findings lend support to the idea that there may be a relative delay in the maturation of cortical inhibitory mechanisms (*cf* Huttenlocher 1967 Meyerson and Persson 1973) The late development of axosomatic synapses, considered inhibitory in nature (*e g* Colonnier 1968) compared to axodendritic ones (Voeller *et al.* 1963 Meller *et al.* 1968 Adinolfi 1971 1972) may serve as further evidence for such a supposition However the significance of axodendritic inhibition in the immature cortex (Purpura 1971) and hippocampus (Purpura, Prelevic and Santini 1968) has been proposed recently It has been claimed that inhibitory postsynaptic mechanisms, both in the cortex and hippocampus show a precocious development compared to excitatory ones (Purpura 1969 1971 1972) The fact that these observations were made on newborn kittens (compare with fetal sheep of 80—90 days of age) implies that no stage was studied sufficient immature to justify a general statement about the relative rate of functional development of excitatory and inhibitory postsynaptic mechanisms in the cortex. Intra cellular recordings of the responses in cortical neurons to peripheral stimulation have to be performed in fetal animals to settle this question.

In recent publications (Scherrer *et al.* 1968, 1970) it has been discussed that the time velocity and flow of neuronal signals are important factors, which contribute to the differences in function of neonatal and mature nervous system. The over-all conduction time from the receptors to the cortex of very young sheep fetuses is long Between 80 and 120 days, there is a rapid and continuous decrease in conduction time. This change is dependent upon several factors such as changes in synaptic transmission efficiency increasing of conduction velocity and lengthening of conduction pathways during growth (*cf* Myslivecek 1968 a) The major factor contributing to the decrease of the onset latency is presumably the increase of afferent fiber diameters and in particular the development of myelin as repeatedly emphasized (references, see Skoglund 1969) In the fetal sheep myelin can be detected in the trigeminal nerve at 60 days of age (Barlow 1969) in the main sensory trigeminal nuclei at 66 days (Romanes 1947) and in the thalamic radiation at 78 days (Barlow 1969) Conduction time approaching adult values was obtained in fetuses of 120 days of age. The fact, that the distance from skin receptors to cortex in these fetuses is short compared to that of the adult animal, implies that the conduction time attains adult

values long before conduction velocity (*cf* Scherrer *et al* 1968) This finding is in accordance with the wellknown fact that the conduction velocities of peripheral (Hursh 1939 Skoglund 1960) and central nerve fibers (Verley 1967 a, Meyerson 1968 b Conway *et al.* 1969) of fetal sheep and of neonatal cats and rabbits are slow The fatiguability of the somatic sensory system is extremely marked in young fetuses, as a stimulus interval of up to 20 sec had to be used to obtain reproducible responses with constant amplitudes. During development there is an increased capacity of the system to mediate closely spaced signals and generate constant electrocortical responses (*cf* Scherrer and Oeconomos 1954 Mollиver 1967) Thus, in the fetal sheep one is confronted with an ineffective somesthetic system in terms of low conduction velocities and reduced flow of the sensory neuronal signals which are considered to be of importance for the sensory feed-back mechanisms and memory processes (Scherrer *et al* 1968 1970)

2 Development of the specific and nonspecific somatosensory systems

The similarity between the somatotopic organization of the ipsilateral upper lip in the immature fetal sheep and in the adult (Adrian 1943 Hatton and Rubel 1967) indicates that the lay out of adult cortical projection is present already at an early developmental stage. The results from a recent study on the cortical somatotopy of newborn and adult cats (Rubel 1971) are in accordance with this finding, since it was found that the projections from all the contralateral body surface to the primary sensorimotor cortex were present at birth and organized in a similar way to that of the adult animal. From the time when the adult pattern of convolutions and fissures can be observed in the fetal sheep the ipsilateral evoked potentials were found to be confined to those areas which correspond to the primary and secondary somesthetic areas in the adult as defined electrophysiologically (Adrian 1943 Woolsey and Fairman 1946) and morphologically (Rose 1942) In no case was it possible to record somesthetic responses from other brain areas The predominately negative evoked response of fetuses between 80—90 days of age is identical to the cortical potential obtained in newborn cat (Purpura 1961 a,b 1962) and rabbit (Verley *et al* 1966 Verley 1967 a, Verley and Siou 1967) in response to electrical stimulation of the specific thalamic relay nuclei. In the adult sheep it has been shown that electrical stimulation applied to the sensory endings of the trigeminal nerve evokes prominent responses in the ipsilateral ventroposteromedial (VPM) nucleus (Richard, Auffray and Albe Fessard 1967 Cabral and Johnson 1971) as well as in the primary somesthetic cortex (Nougier 1963) In view of these findings, it is assumed that the cortical response evoked by tactile stimulation of the

ipsilateral trigeminal nose region in sheep fetuses during development results essentially from an activation via the specific thalamocortical system. A similar conclusion about the trigeminal afferent input to the somatic sensory area of newborn rabbit has been reported (Verley 1967 b)

It is a wellknown fact that in the adult unanesthetized animal different types of sensory stimuli may evoke responses within the so-called association areas of the cortex (*e.g.* Buser and Bignall 1967 Thompson, Bettinger Birch and Groves 1969) The failure to record such responses to trigeminal tactile stimulation in any of the fetuses indicates a relative retardation of this associative somatosensory system. It is of interest in this connection to refer to a recent study by Mayers, Robertson Rubel and Thompson (1971) They demonstrated that in postnatal kittens, sensory evoked unit responses in association areas developed comparatively late and that the somatosensory units appeared later than visual and auditory ones.

A cortical afterdischarge triggered by single sensory stimulation has been observed in the somesthetic (Scherrer and Oeconomos 1954) and visual cortex (Marty 1962 Huttenlocher 1967 Rose *et al* 1972) of unanesthetized postnatal kittens. The findings in the present study that the cortical after discharge appeared later in development than the primary' evoked response, and that it was selectively suppressed by low frequency repetitive stimulation support the view that these two cortical activities are generated by different subcortico-cortical mechanisms. Furthermore it was frequently observed that cortical neurons fired solely concomitantly with either of the two electrocortical activities. There is reason to believe that the appearance during development of the cortical afterdischarge is dependent on the functional maturation of nonspecific reticular and thalamic structures and their integration with the afferent specific system. Firstly the simultaneous appearance and the similar pattern of the evoked cortical afterdischarge and spontaneous EEG-spindles (Bernhard *et al* 1959 Bernhard and Meyerson 1968 Meyerson 1968 b) may indicate a common subcortical drive for these electrocortical activities. The importance of intrathalamic integration for the synchronization of the thalamocortical rhythmic activities in the adult (references, see Andersen and Andersson 1968 Purpura 1968 1972) and immature animal (Thatcher and Purpura 1972) has been stressed Secondly since the flash-evoked cortical afterdischarge observed in postnatal kittens develops into a desynchronizing activity typical of adult electrocortical arousal it has been considered to constitute a primitive reticular arousal (Rose *et al.* 1972)

On the basis of the foregoing discussion, it seems likely that there is a differential development of specific and nonspecific afferent systems projecting to the *somatosensory* cortical areas from the trigeminal region The

specific pathways may activate the cerebral cortex already during early prenatal stages as shown by the presence of the primary evoked response whereas generalized and associative activation involving the ascending reticular system integrated with the specific afferent system, seems to appear comparatively late during ontogeny

In this context it is of relevance to make a comparison with the differential development of the specific and nonspecific components of the visual and auditory systems. It has recently been demonstrated in the prenatal sheep that the development of visually evoked cortical responses occurs in two phases (Persson and Stenberg 1972) A similar developmental course has previously been described in the postnatal development of cat, dog and rat (Marty *et al.* 1959 Marty 1962 Fox 1968 Mysliveček 1968 a Rose 1968 a,b 1971 Rose and Lindsley 1968) Based on the different reactions to selective subcortical lesions of the different components of the cortical response it has been proposed that the early appearing negative component is mediated via the nonspecific optic projection. Components appearing later in development have been considered to be dependent on the specific visual pathways (Rose and Lindsley 1968) On the basis of studies on auditory evoked electrocortical responses in postnatal dogs to electrical stimulation in the periphery and in the medial geniculate body it has been assumed that the specific thalamic relay nucleus is indispensable for evoked auditory responses in the newborn animal (Myslivček 1968 b)

3 *Functional development of the somatosensory cortex and its morphological correlates*

Earlier investigations on the postnatal development of evoked surface responses in sensory cortex in various species have demonstrated the presence of a predominant surface-negative potential immediately after birth. The precocity of the superficial axodendritic neuropile has been considered to account for this feature of the evoked potential (Marty *et al* 1961 Scheibel 1962 Marty and Scherrer 1964 Purpura *et al* 1964) However the characteristic surface positive wave form of the somatosensory evoked response in immature fetal sheep suggests a different mode of electrocortical activation in the cortex during early prenatal stages of ontogeny This is not due to species-specific organization of the sheep's cortex, since it has been reported that predominantly positive evoked cortical responses may be obtained also in the cortex of fetal dog (Molliver personal communication) In addition, somatosensory evoked cortical responses displaying initial positivity have occasionally been recorded in newborn rats (Thairu 1971)

The results of the laminar recordings of both gross and unit evoked activity

ipsilateral trigeminal nose region in sheep fetuses during development results essentially from an activation via the specific thalamocortical system. A similar conclusion about the trigeminal afferent input to the somatic sensory area of newborn rabbit has been reported (Verley 1967 b).

It is a wellknown fact that in the adult unanesthetized animal different types of sensory stimuli may evoke responses within the so-called association areas of the cortex (e.g. Buser and Bignall 1967, Thompson, Bettinger, Birch and Groves 1969). The failure to record such responses to trigeminal tactile stimulation in any of the fetuses indicates a relative retardation of this associative somatosensory system. It is of interest in this connection to refer to a recent study by Mayers, Robertson, Rubel and Thompson (1971). They demonstrated that in postnatal kittens sensory evoked unit responses in association areas developed comparatively late and that the somatosensory units appeared later than visual and auditory ones.

A cortical afterdischarge triggered by single sensory stimulation has been observed in the somesthetic (Scherrer and Oeconomos 1954) and visual cortex (Marty 1962, Huttenlocher 1967, Rose *et al.* 1972) of unanesthetized postnatal kittens. The findings in the present study that the cortical afterdischarge appeared later in development than the primary evoked response and that it was selectively suppressed by low frequency repetitive stimulation support th[illegible]hat these two cortical activities are generated by different subcortico[illegible] mechanisms. Furthermore it was frequently observed that cortical neu[illegible]fired solely concomitantly with either of the two electrocortical activities. There is reason to believe that the appearance during development of the cortical afterdischarge is dependent on the functional maturation of nonspecific reticular and thalamic structures and their integration with the afferent specific system. Firstly, the simultaneous appearance and the similar pattern of the evoked cortical afterdischarge and spontaneous EEG-spindles (Bernhard *et al.* 1959, Bernhard and Meyerson 1968, Meyerson 1968 b) may indicate a common subcortical drive for these electrocortical activities. The importance of intrathalamic integration for the synchronization of the thalamocortical rhythmic activities in the adult (references, see Andersen and Andersson 1968, Purpura 1968, 1972) and immature animal (Thatcher and Purpura 1972) has been stressed. Secondly, since the flash-evoked cortical afterdischarge observed in postnatal kittens develops into a desynchronizing activity typical of adult electrocortical arousal it has been considered to constitute a primitive reticular arousal (Rose *et al.* 1972).

On the basis of the foregoing discussion, it seems likely that there is a differential development of specific and nonspecific afferent systems projecting to the *somatosensory* cortical areas from the trigeminal region. The

since its net current sink is located in the primordium of layer IV and since the concomitant single unit activity is encountered at the same cortical depths. A small fraction of the surface positivity might be of a presynaptic origin as shown by its ability to follow repetitive stimulation It has been assumed that superficial inhibitory cortical activation contributes to positive surface potentials in the adult cortex (Purpura 1959 Creutzfeldt *et al* 1966 a) It is less likely that in young sheep fetuses superficial inhibition substantially contributes to the positive component of the surface response since topically applied strychnine has no effect.

At the same developmental stage the negative component of the evoked surface response corresponds to a deep current sink-source pair at the lower border of the cortex but no concomitant single unit activity can be recorded. A subthreshold excitatory drive on the deep cortical neurons without spike generation may be suggested, since it has been assumed that immature cortical neurons exhibit a *relatively high level* of postsynaptic excitability but a strikingly low level of spike responsiveness (Purpura 1972) However the possibility of an inhibitory activation of neuronal elements situated at the lower cortical border cannot be disregarded. There is no experimental evidence of a synaptic activation of neurons in the superficial cortical strata.

Histological investigations of the cortex of fetal sheep during this developmental stage demonstrates the presence of fairly well-defined lamination appearing as 5 layers of alternating high and low cell density (*cf* Åström 1967) Layers with low cell density have been considered to be caused by separation of neurons due to the formation of dendrites and axons (Lorente de Nó 1933 Åström 1967) There is a good correlation between the cortical depths from which evoked activity was obtained and the depths of the low cell density layers, which presumably contain the morphological pre- and postsynaptic substrate for the activity

The afferent fibers *reaching the cortex* at *this developmental stage* are directed towards the marginal and midcortical layers (Åström 1967) In recent studies on the fetal cat brain during a comparable developmental stage it has been shown that a new set of afferent fibers, assumed to be specific, pen trates into the lower region of the pyramidal layer (Marin Padilla 1971 1972) The apical dendrites with signs of developing shaftspines and the well-developed basal dendrites of the deep pyramidal neurons and the mature stellate cells, present in deep cortical layers (IV—VI Åström 1967) may serve as postsynaptic elements. Of special interest is the appearance of spines on the apical shafts of the deep pyramids since they have been considered to constitute a main postsynaptic locus for the afferent fibers of the specific thalamic nuclei to the somesthetic (Jones and Powell 1970) as well as to the

visual cortex of the adult animal (Globus and Scheibel 1967 a,b Garey and Powell 1971) In a recent electron microscopic study of the vertical distribution of synapses in the neocortex of newborn dog (Molliver and van der Loos 1970) three regions of high synaptic density were found one corresponding to the marginal layer and two corresponding to the deep layers containing the large pyramidal neurons. On the basis of the functional data presented by Molliver it was assumed that synaptic functions were first established in the deep cortical strata. This idea harmonizes with the results of the present study It should be added that the evolvement in deep layers of recurrent axonal collaterals and stellate cells in the cortex of fetal sheep may provide the morphological substrate for intracortical connections. The functional role of stellate cells, which in the mature neocortex amount to 25% of total population of nerve cells, has been emphasized (Scheibel 1962 Schadé Backer and Colon 1964)

During the following developmental period (75—90 days) in fetal sheep there is a transformation of the surface response from a biphasic positive negative to a monophasic negative form. This alteration is combined with an increasing amplitude and decreasing latency of the negative component which thus seems to mask and eventually extinguish the initial positive wave. As mentioned earlier the monopolar negative response in the fetal sheep is identical to that obtained in neonatal altricial animals with peripheral somesthetic stimulation (*e.g* Scherrer and Oeconomos 1954 Marty 1962 Thairu 1971) The observations of net current sinks corresponding to the surface positivity and negativity in cortical layers I—III suggest the presence of superficial excitatory' generators. This interpretation is substantiated by the presence of evoked unit activity in these same layers. In addition the laminar potential data suggest that the surface response receives a greater contribution from the neuronal activity in superficial cortical layers than from the activity in the deeper layers. In recent studies on sensory evoked responses in the somesthetic cortex of newborn rabbits (Verley and Rokyta 1972) and in the auditory cortex of postnatal cats (König, Pujol and Marty 1972) similar results have been reported The observations that during this developmental stage strychnine alters the form and amplitude of the evoked surface response and that peripheral stimulation may inhibit spontaneously active cortical neurons lend support to the view that inhibitory phenomena may play an important role in the cortex. This notion is compatible with the demonstration of prominent and prolonged IPSP s in cortical (Purpura *et al* 1965) and hippocampal neurons of newborn cats (Purpura *et al* 1968)

There are several morphological characteristics which may account for the data indicating a functional maturation of the superficial layers of the

neocortex during this developmental period (75—90 days) Thus, the immature bipolar cortical neurons in layers II—III attain their mature form (Åström 1967) The successive evolvement of apical and in particular basilar dendrites of the pyramids in these layers, indicates that this stage constitutes the formative phase of the postsynaptic elements in the superficial neuropile. Electron microscopic investigations of the neocortex of fetal and newborn cat have shown a characteristic abundance of axodendritic synapses in the superficial strata at a corresponding developmental stage (Voeller *et al.* 1963 Adinolfi 1971 1972) Furthermore, in a synapto-architectonic study on the somesthetic cortex of the newborn dog a high level of synaptic density was observed in the marginal layer containing the Retzius-Cajal cells and the terminal arborizations of the apical dendrites (Molliver and van der Loos 1970) A spread of neuronal activity can be mediated from deep to superficial strata by the way of recurrent collaterals of the deep pyramids and interneurons. The presence of elaborate interneurons is of particular interest since cells of this class (*cellule à double bouquet dendritique de Cajal* Cajal 1955) have been assumed to be responsible for the vertical spread of excitation in the vertical columns of the adult cortex (Colonnier 1964) In view of observations made in fetal and newborn cat (Marin-Padilla 1972, Laemle, Benhamida and Purpura 1972) one may also assume that in fetal sheep displaying surface negative responses, afferent fibers directly activate the superficial layers.

In the present investigation the results obtained from the laminar analysis of evoked field potentials and from the vertical distribution of evoked unit discharges reveal that during the early prenatal ontogenesis of the sheep the trigeminal afferent inflow initially activates neuronal elements in the immediate subcortical stratum. During subsequent development the activation takes place in the deep layers of the cortex and successively invades superficial layers. This corticopetal gradient of functional development is expressed by the gradual alterations in the configuration of the evoked response from a surface positivity to a surface negativity It is relevant to refer to findings on th developing visual (Persson and Stenberg 1972) and transcallosal cortical responses (Meyerson 1968 b) in fetal sheep. At their earliest appearance these responses display a predominating surface positive wave and subsequently undergo changes similar to those of the somesthetic response These observations suggest that, in general, the functional development of the cortex takes place along a corticopetal gradient. The morphological correlation to this pattern of functional development is the well documented observation that, in general the maturation of the neurofibrillar organization in the cortex of mammals, takes place along a corticopetal gradient from

deep to superficial strata (Vignal 1888 Lorente de Nó 1933 Cajal 1960 Angevine and Sidman 1961 Berry and Rogers 1965 Caley and Maxwell 1968 for discussion, see Molliver and van der Loos 1970)

The beginning of the final developmental period is marked by the reappearance of *a small initial* positivity in the surface response. Later the positivity increases in amplitude and the response attains the adult positive-negative configuration. The reappearance of the positive component during late prenatal development is paralleled by marked changes of the temporal relationship of the net current sources and sinks. The deep sink observed in previous stages is now recorded at an earlier phase of the cortical activation and this in turn gives rise to the initial surface positivity Thus, the well-known transformation during development of the evoked cortical potentials from a surface negativity to a surface positive-negative form may tentatively be interpreted as being the result of an unmasking of a pre-existing excitation located in the lower part of the cortex. It is interesting to note that the deep and superficial centers of neuronal activity generating the positivity and negativity of the developing cortical response in fetal sheep resemble the A and B-generators described in adult cats (Calvet, Calvet and Scherrer 1964 Calvet and Calvet 1965 Calvet, Calvet and Langlois 1965) and in newborn rabbits (Garma and Verley 1965 1967 Verley 1965 1968) On the basis of the results described one may assume an early net excitatory activation of neurons in the midcortex followed by the spread of activity to superficial strata much in the same way as has been suggested for the adult somesthetic cortex (Eccles 1964 Towe 1966 Landau 1967)

From studies in postnatal animals it has been proposed (Scheibel 1962 Scheibel and Scheibel 1964 1971) that the evolvement of the neuropile in layer IV with regard to specific corticopetal fibers and stellate cells may account for the reappearance of the surface positive wave. Alternatively the significance of a further development of basal dendrites of the deep-seated pyramids together with the development of axosomatic synapses has been emphasized (Marty *et al* 1961 Marty 1962 Marty and Scherrer 1964 Purpura *et al.* 1964) The details of the later morphological maturation of the neocortex of fetal sheep are still lacking However well developed basal dendrites in conjunction with the deep pyramids are present in fetal sheep at an earlier stage, and it is therefore likely that the development of the neuropile of the layer IV constitutes a major factor for the reappearance of the surface positivity

SUMMARY

The prenatal development of somatosensory cortical functions was investigated by recording evoked surface and depth gross responses as well as extracellular single unit activity following tactile stimulation of the trigeminal nose area. Experiments were performed on externalized and nonanesthetized sheep fetuses with ages from 42 days to full term kept in contact with the decerebrate ewe through the intact umbilical cord.

Laminar analyses of evoked field potentials and depth-distributions of evoked single unit responses showed that with age there was a change of the locus of activity in the somesthetic cortex which reflected alterations in the configuration of the surface response. The functional maturation of the somatosensory system was further denoted by changes of the evoked surface potential with respect to its cortical distribution latency amplitude and ability to follow repetitive stimulation occuring as a function of age.

During the developmental period between 42—68 days the evoked surface response consisted of a long latency positive wave. Negative evoked field potentials and evoked single unit responses were obtained only in the subcortical strata of the telencephalic wall Whether or not this single unit activity is of pre or postsynaptic origin cannot be definitely stated No signs of intracortical activation could be observed Thus, the results indicate that during early ontogeny the trigeminal somatosensory system operates as a dead-end afferent system with no functional contacts with the cortex proper

During the developmental stage between 68—75 days the evoked surface response had a biphasic positive-negative configuration with a predominating positivity Both the laminar analysis of the evoked potentials as well as the depth-distribution of the evoked single unit activity showed that around 68—70 days the tactile stimulation activated the cortex for the first time The evoked cortical activity was present in the primordia of cortical layers IV—VI and there were no signs of activation of superficial strata. The cortical neurons responded with a single spike regardless of the strength and of the location of the stimulus within the peripheral receptive field Correlative morphological data indicate that afferent fibers shown to reach the mid pyramidal layers may constitute the presynaptic substrate for the cortical

activation The dendrites of the deep pyramids and the mature stellate cells, present in the deep cortical layers (IV—VI) may serve as postsynaptic elements.

During the subsequent developmental period (75—90 days) the surface response underwent alterations in configuration from a predominantly positive to a monopolar negative form. The surface negative response corresponded to negative field potentials obtained in all cortical layers. Unit responses from neurons activated by the trigeminal inflow could now be recorded throughout the cortex. Correlative morphological data indicate that this stage constitutes the formative phase of the postsynaptic structures in the superficial neuropile. A spread of neuronal activity from deep to superficial strata may be mediated by way of intracortical connections through interneurons and recurrent collaterals of the deep pyramids as well as of corticopetal afferent fibers directed towards the superficial neuropile.

The laminar analysis of evoked field potentials and the vertical distribution of responding units show that at the end of the first trimester the trigeminal inflow activates neuronal elements in the immediate subcortical stratum. During the following development the activation takes place in deep layers of the cortex and later on also invades more superficial strata. This corticopetal gradient of functional development is expressed by the gradual alterations in the configuration of the evoked surface response from a posi tivity to a negativity

On the basis of the selective effects of topical strychnine on the developing somesthetic surface response it is assumed that there is a delayed development of inhibitory postsynaptic mechanisms in the cortex. Arrest of spontaneously discharging cortical units as a result of trigeminal stimulation was not possible until a fetal age of about 90 days.

The representation of the ipsilateral upper lip in the cortex of immature fetal sheep was similar to that of the adult When the adult pattern of convolutions and fissures could be traced in the fetal brain the response was confined to those areas which correspond to the primary and secondary somesthetic areas of the adult sheep Responses in the cortical associative areas were never obtained In some older fetuses (85—120 days) the response also displayed a long-latency afterdischarge following the initial primary complex. The finding that the primary' response and the afterdischarge showed selective reactions to repetitive stimulation and were accompanied by single unit activity of different characteristics indicates that these two electrocortical activities are generated by different subcortico-cortical mechanisms. On the basis of these findings it has been suggested that the specific and nonspecific afferent inputs to the somatosensory cortex have a

differential rate of maturation.

From a fetal age of around 100 days, the evoked surface response changed from a monopolar negative to a positive negative form similar to that of the adult. The results from the laminar potential analysis lend support to the view that the reappearance of the surface positivity results from an unmasking of a pre-existing excitatory generator located in deep cortical layers. In these later stages of development the cortical neurons were capable of responding with repetitive discharges to tactile stimulation The observations of the present study suggest that, in the perinatal sheep the somatosensory cortex has reached a comparatively high degree of functional maturation.

ACKNOWLEDGEMENTS

This investigation has been supported by grants to the Department of Physiology II from the Association for the Aid of Crippled Children, Magnus Bergvalls Stiftelse and from the Swedish Medical Research Council by personal grants from Svenska Sällskapet för Medicinsk Forskning, Karolinska Institutets Fonder and Hierta Retzius' Fond för Vetenskaplig Forskning

I wish to express my sincere gratitude to Professor Carl-Gustaf Bernhard and to Docent Björn Meyerson for their invaluable and active help during all parts of this work.

REFERENCES

Adinolfi, A. M. The postnatal development of synaptic contacts in the cerebral cortex. I M. B. Sterman, D J McGinty and A. M. Adinolfi (Eds.) *Brain Development and Behavior* Academic Press, New York 1971 73—89

Adinolfi, A. M. Morphogenesis of synaptic junctions in layers I and II of the somatic sensory cortex. *Exp Neurol.* 1972. *34* 372—382

Adolph E. F., Physiological stages in the development of mammals. *Growth* 1970. *34* 113—124

Adrian, E. D Afferent areas in the brai of ungulates. *Brain* 1943 *66* 89—103.

Allison, T Recovery cycles of primary evoked potentials in cat sensorimotor cortex. *E perientia (Basel)* 1968. *24* 240— 41

Amassian, V E. Microelectrode studies of the cerebral cortex. *I t Rev Neurobiol* 1961 *3* 67—136.

Amassian, V E., H. D Patton, J W Woodbury A. Towe and J E. Schlag An inter pretation of the surface response in somatosensory cortex to peripheral and interareal afferent stimulation. *Electroe ph I n. N rophysiol.* 1955 *7* 480—483

Amassian, V E. H J Waller and J Macy J., Neural mechanism of the primary somatosensory evoked potential. *A n. N Y Acad Sci.* 1964 *112* 5—32

Andersen, P and S A. Andersson, *Physiologic l Basis f th Alpha Rhytm.* Appleton Century and Crofts, New York 1968

Andersen P and D R Curtis, The pharmacology of the synaptic and acetylcholine-induced excitation of ventrobasal thalamic neurones. *A t physiol. sc d* 1964 *61* 100—120

Angel, A and O Holmes, U itary nd mass cortical potentials evoked by peripheral stimulation of anaesthetized rats. *Nat (Lo d)* 1967 *214* 834—835.

Angevine J B. and R. L. Sidman, Autoradiographic study of cell migration during histogenesis of cerebral cortex in the mouse. *Nat (Lond)* 1961 *192* 766—768.

X ggård L An lectrophysiological study of the development of cochlear functions in the rabbit. *A ta to-laryng (Stockh)* 1965 Suppl. 203

Ånggård L., R. Bergström and C. G Bernhard, Analysis of prenatal spinal reflex activity in sheep. *A t physiol and.* 1961 *53* 128—136

Ånggård, L. and D Ottoson, Observ tions on the functional development of the neuromuscular pparatus in fetal sheep. *E p Ne ol.* 1963 *7* 294—304

Armstrong-James, M A., Spontaneous and evoked single unit activity in seven-day rat cerebral cortex. *J Physiol. (Lo d)* 1970. *208* 10P

Aström, K. E., On the early dev lopment of isocortex i fetal heep. In C. G Bernhard and J P Schadé (Eds.) *Progr Brain Resea h* Vol. 26 *D velopmental N urology* Elsevier Amsterdam 1967 1—59

Barcroft, J and D H. Barron, Movement in the mammalian foetus. *Ergebn. Physiol.* 1939 *42* 107—152

Barcroft, J and D H. Barron. The dev lopment of behavior in foetal sheep *J omp. Neu ol* 1939 b. *70* 477— 502

Barcroft, J and D H. Barron, Observations on the functional development of the foetal brain. *J comp Neurol.* 1912. 77 431—454

Barcroft, J and D H. Barron, Blood pressure and pulse rate in foetal sheep. *J e p Biol.* 1945. 22 63—74

Barlow R. M., The foetal sheep morphogenesis of the nervous system and histochemical aspects of myelination. *J comp Neurol.* 1969 *135* 249—262.

Barlow H. B. and J D Pettigrew Lack of specificity of neurones in the visual cortex of young kittens. *J Physiol. (Lo d)* 1971 *218.* 98—100P

Barron, D H The functional development of some mammalia neuromuscular mechanisms. *Biol Rev* 1941 *16* 1—33

Bergström, R. C. G Bernhard and L. Änggård, Analysis of some prenatal reflex functions in sheep. *A ta physiol. s and* 1960. *50* 23—24

Bernhard, C. G I H Kaiser and G M Kolmodin On the development of cortical activity in fetal sheep. *Acta physiol. cand* 1959 *47* 333—349

Bernhard, C. G I. H. Kaiser and G M. Kolmodin, On the epileptogenic properties of the fetal brain. *Acta Paed at (Uppsala)* 1962. *51* 81—87

Bernhard, C. G G M. Kolmodin and B. A Meyerson, On the prenatal development of function and structure in the somesthetic cortex of the sheep In C. G Bernhard and J P Schadé (Eds.) *Progress in B ain Rese h* Vol. 26 *Developmental Neurology* Elsevier Amsterdam 1967 60—77

Bernhard C. G and B. A. Meyerson, Early ontogenesis of electrocortical activity With special reference to experimental studies on fetal sheep. In P Kellaway and I. Petersén (Eds.) *Clinical Electroen phalography of Children* Almqvist and Wiksell Stockholm Grune and Stratton, New York 1968 11—29

Bernhard, C. G and B. A. Meyerson Electrophysiological aspects on the development of recipient functions in the cerebral cortex. In *J Barcroft C tenary Symposi m on Prenatal and Neonatal Physiology* Cambridge Univ Press, Cambridge 1973 (In press)

Bernhard, C. G B. A Meyerson and H. E. Persson, Etudes électrophysiologiques de l'ontogenèse précoce des fonctions réceptrices sein d cortex sensori-moteur *A tualités europhysiol* 1972. 2 119—144

Berry M. and A. W Rogers, The migration of neuroblasts in the developing cerebral cortex. *J Anat. (Lond)* 1965 *99* 691—709

Borbély A. A., Changes in click-evoked responses as function of depth in auditory cortex of the rat. *B ain Res* 1970. *21* 217—247

Bremer F Cerebral and cerebellar potentials. *Physiol. Re* 1958 *38.* 357—388.

Briquel, F and R. V rley Le développement fonctionel d système somesthésique étudié par double stim lation chez le lapin nouveau-né. *A h. sci. physiol.* 1972. *26* 303—312

Buser P and K. E. Bignall, Nonprimary sensory projections on the cat neocortex. *Int Rev Neurobiol.* 1967 *10* 111—165.

Cabral, R. J and J J Johnson, The organization of mechanoreceptive projections i the entrobasal thalamus of sheep. *J mp Neurol.* 1971 *141* 17—35

Cajal, Ramón y S., *Histologie d Systèm Nerveux* V I. II Instituto Ramón y Cajal, M drid 1955

Cajal, Ramón y S., *Studies Vertebrate Neurogenesis.* (transl. L. Guth) Charles C. Thomas, Springfield 1960

Caley D W and D S. Maxwell An electron microscopic study of neurons during postnatal development of the rat cerebral cortex. *J comp Neurol.* 1968. *133* 17—44

Calvet, J nd M. C. Calvet, Générateurs corticaux responsables des réponses augmentantes et recruta tes. *J Physiol. (Paris)* 1965. *57* 503—510.

Calvet, J M C. Calvet and J M. Langlois, Diffuse cortical activation waves during so-called desynchronized EEG patterns. *J Ne rophysiol.* 1965. *28* 893—907

Calvet, J M. C. Calvet and J Scherrer Etude stratigraphique corticale de l'activité EEG spontanée *Ele troenceph clin Neu physiol* 1964 *17* 109—125.
Carmichael, L. Ontogenetic development. In S. S. Stevens (Ed.) *H ndbook of Ex perimental Psychology* Wiley New York 1951 281—303
Carmichael, L., The onset and early development of behavior In L. Carmichael (Ed.) *Man al f Child Psychology* J Wiley and Sons London 1954 60—185.
Carter M. C., O Holmes and J Houchin Spatial distribution of lectrical potentials evoked in the cerebral cortex of rats by peripheral stimulation. *J Physol. (Lond)* 1968 *201* 16—18P
Clawson, A. B., Normal rectal temperat res of sheep. *Amer J Physiol* 1928, *85* 251—270
Colonnier M L., The structural design of the neocortex. In J C. Eccles (Ed.) *Brain and C nscious Experie ce* Springer Verlag New York 1964 1—23
Colonnier M L. Synaptic patterns of different cell types in the different laminae of the cat visual cortex. An electron microscope study *Brai Res.* 1968 *9* 268—287
Conway C. J F S. Wright and W Bradley Electrophysiological maturation of the pyramidal tract in the postnatal rabbit. *Electroenceph. clin. N u ophysiol.* 1969 *26* 565—577
Creutzfeldt, O D Neuronal mechanisms underlying the EEG I H. H. Jasper A. A. Ward and A Pope (Eds.) *Basic M cha isms of the Epil psies* Little, Brown Boston 1969 397—410.
Creutzfeldt, O D A Rosina, M. Ito and W Probst Visual evoked response of single cells and of the EEG in primary visual area of the cat *J Neurophysiol.* 1969 *32* 127—139
Creutzf ldt O D S Watanabe and H. D Lux, Relations between EEG phenomena and potentials of single cortical cells. I Evoked responses after thalamic and epicortical stimulation. *Elect oe eph. cli Neurophysiol.* 1966 a. *20* 1—18
Creutzf ldt O. D S. Watanabe and H D Lux R lations between EEG phenomena and potentials of single cortical cells. II Spontaneous and convulsoid activity *Electroenc ph cli Neu physiol.* 1966 b. *20* 19—37
Cross, K. W G S. Dawes nd J C. Mott, Anoxia, oxygen consumption and cardiac output in new-born lambs and adult sheep. *J Physol. (Lond)* 1959 *146* 316—343
Darian-Smith, I Neural mechanisms of facial sensation. *I t R v Neurobiol.* 1966. *9* 301—395
Delhaye-Bouchaud, N *Et d d l'Evolutio d P tential Evoq é Somesthé iqu che le Lapi pendant la Périod Néonatal* Thèse Paris 1964
Eccles, J C., I terpretation of action potentials evoked in th cerebral cortex. *Ele t oe ph. cli Neu physol.* 1951 *3* 449—464
Eccles, J C. Cerebral synaptic mechanisms. In J C. Eccles (Ed.) *Brai and Conscious E perie* Springer Verlag, New York 1964 24—58.
Eidelberg, E. G M Kolmodin and B. A. Meyerson Ontogenesis of steady potential and direct cortical response in fetal sheep brain. *Exp Neurol.* 1965. *12* 198—214
Eidelberg, E. G. M Kolmodin and B. A Meyerson Effect of asphyxia on the cortical steady potential in adult and fetal sheep. *A t physiol scand* 1967 *69* 257—261
Ekholm, J Postnatal changes in cutaneous reflexes and in the discharge pattern of cutaneous and articular sense organs. *Act physiol. cand* 1967 Suppl. 297
Ellingson, R. J and R C. Wilcott, Development of evoked responses i visual and auditory cortices of kittens. *J N urophysiol* 1960 *3* 363—375.
v E ler C. and G F Ricci, Cortical evoked responses in auditory area and significance of apical dendrites. *J Neurophysiol* 1958 *21* 231—246.
Fox, M. W Neuronal development and ontogeny of evoked potentials in auditory and visual cortex of the dog *Electroe c ph l N physol* 1968. *24* 213—226.
Galindo A K. Krnjević and S. Schwartz, Patterns of firing in cuneat neurones nd

some effects of flaxedil. *Exp. Brain Res.* 1968, 5 87—101
Garey L. J and T P S. Powell An experimental study of the termination of the lateral geniculo-cortical pathway in the cat and monkey *Proc roy Soc B (Lond)* 1971 *179* 41—63.
Garma, L. and R. Verley Générateurs corticaux étudiés par électrodes implantées hez le lapin nouveau-né. *J Physiol. (Paris)* 1965. *57* 811—818.
Garma. L. and R. Verley Activités cellulaires corticales étudiées par électrodes implantées chez le lapin nouveau-né. *J Physiol (Paris)* 1967 *59* 357—376.
Globus, A. and A B Scheibel, Synaptic loci on visual cortical neurons of the rabbit The specific afferent radiation. *Exp Neurol.* 1967 *18* 116—131
Globus, A. and A. B. Scheibel, The effect of visual deprivation on cortical neurons A Golgi study *Exp Neurol.* 1967 b *19* 331—345
Gottlieb G Ontogenesis of sensory function in birds and mammals. In E. Tobach L. R. Aronson and E. Shaw (Eds.) *Th Biopsychology of Devel pment* Academic Press, New York 1971 67—128.
Grafstein, B., Postnatal development of the transcallosal evoked response in the cerebral cortex of the cat. *J Neurophysiol.* 1963 *26* 79—99
Grossman, C., Electro-ontogenesis of cerebral activity *Arch. Neurol. Psychiat (Chic)* 1955 *74* 186—202.
Grossman, R. G L. Whiteside and T L. Hampton, The time course of evoked depolarization of cortical glial cells. *Brain Res.* 1969 *14* 401—415
Halpern, L. M. and R. G Black, Flaxedil (Gallamine triethiodide) E idence for a central action. *Science* 1967 *155* 1685—1687
Hatton, G. I od E. W Rubel Somatic sensory projections in cerebral cortex of sheep. *A at Rec* 1967 *157* 256—257
H bel, D H. and T N Wiesel, Receptive fields of cells in striate cortex of very young, visually inexperienced kittens. *J Neurophysiol.* 1963 26 994—1003.
Humphrey D. R., Re-analysis of the antidromic cortical response. I Potentials evoked by stimulation of the isolated pyramidal tract. *Electroenceph. clin N ur physiol.* 1968 a. 24 116—129
Humphrey D R., Re-analysis of the antidromic cortical response II On the contribution of cell discharge and PSPs to the evoked potentials. *Electroen ph. clin. Neurophysiol.* 1968 b. *25* 421—442.
Hunt, W E. and S. Goldring, Maturation of evoked response of the visual cortex in the postnatal rabbit. *Elect oenceph. clin N urophysiol.* 1951 *3* 465—471
Hursh J B Th properties of growing nerve fibers. *Amer J Physiol.* 1939 *127* 140—153
H ttenlocher P R., Development of cortical neuronal ctivity in the neonatal cat. *Exp Neurol.* 1967 *17* 247—262.
Hyvärinen, J Analysis of spontaneous spike potential activity in developing rabbit diencephalon. *Acta physiol. scand.* 1966 Suppl. 278.
Jones, E. G and T P S Powell, An electron microscopic study of the laminar pattern and mode of termination of afferent fibre pathways in the somatic sensory cortex of the cat. *Phil. T ans B* 1970 *257* 45—62
Kasprzak, H. D N T pper and H. Craig, Functional development of the tactile pad receptor system. *Exp Neurol.* 1970. *26* 439—446
Kolmodin, G M. and B. A Meyerson, Ontogenesis of paroxysmal cortical activity in foetal sheep. *Electroenceph. clin. Neurophysiol.* 1966. *21* 589—600
König, N R. Pujol and R. Marty A laminar study of evoked potentials and unit responses in the uditory cortex of postnatal cat. *Brain R* 1972. *36* 469—473
Laemle L., C. Benhamida and D. P Purpura, Laminar distribution of geniculocortical aff rents in visual cortex of the postnatal kitten. *Brain Res* 1972 *41* 25—37
Laget, P M A. Thomson and N Delhaye Bouchaud, Activités unitaires corticales,

hippocampiques et cérébelleuses chez le jeune lapin Quelques effets de la maturation. *C R. Soc Biol. (Paris)* 1967 *161* '44—248

Landau W M., Evoked potentials. In G C. Quarton T Melnechuk nd F O. Schmitt (Eds.) *The Neurosciences* Rockef.ller Univ Press, New York 1967 469—482.

Li C. L., C. Cullen and H H. Jasper Laminar microelectrode studies of specific somatosensory cortical potentials. *J N rophysiol.* 1956 *19* 111—130

Lorente de Nó R., Studies on the structure of the cerebral cortex. *J Psychol. Neurol. (Lpz.)* 1933 *45* 381—438.

Marin-Padilla, M., Early prenatal ontogenesis of the cerebral cortex (Neocortex) of the cat (Felis Domestica) A golgi study I The primordial neocortical organization. *Z Anat. Entwickl.-Gesch* 1971 *134* 117—145.

Marin-Padilla, M. Prenatal ontogenetic history of the principal neurons of the neocortex of the cat (Felis domestica) A golgi tudy II Developmental differences and their significances. *Z A at Entwickl.-Gesch* 1972. *136* 125—142.

Marty R., Développement post-natal des réponses sensorielles d cortex cérébral chez le chat et le lapin. Aspects physiologiques et histologiques. *A ch A at micr Morph. e p* 1962. *51* 129—264

M rty R., J Chevrea and J Scherrer M turation des réponses visuelles et édification du neuropile cortical. *C R Soc Biol. (Paris)* 1961 *155* 705—706

Marty R., F Contamin nd J Scherrer La double-réponse électrocorticale à la stimulation lumineuse chez le chat nouvea né. *C R Soc Biol (Paris)* 1959 *153* 198—201

Marty R. and J Scherrer Critères de maturation des systèmes afférents corticaux. In D P Purpura and J P Schadé (Eds.) *Progr ss in Brain Resear h* Vol. 4 *G owth d Mat ration f th Brain* Elsevier Amsterdam 1964 222—234

Marty R. and J Thomas, Réponse électro-corticale à la stimulation d nerf cochléaire hez le chat nouveau-né. *J Physiol. (Paris)* 1963 *55* 165—166

Mayers, K. S R. T Robertson E. W R bel and R. F Thompson, Development of polysensory responses in association cortex of kitten. *Sc ence* 1971 *171* 1038—1040.

Meller K., W Breipohl and P Glees, Synaptic organization of the molecula and the outer granular layer in th motor cortex in the white mouse d ring postnatal development. *Z Zellforsch* 1968 *92* 217—231

Meyerson B A., Electrophysiological signs of interhemispheric functions during development. In L. Jílek and S. Trojan (Eds.) *O tog ens of the B ai* Charles U iv Prague 1968 a. 73—83

Meyerson, B. A., Ontogeny of interhemispheric functions. *A ta physiol. d* 1968 b. Suppl. 31..

Meyerson B. A and H. E Persson, Evoked unitary nd gross electric ctivity in the cerebral cortex in early prenatal ontogeny *Natu (Lond)* 1969 *221* 1248—1249

Meyerson, B. A. and H E. Persson, Early epegenesis of recipient functions in the neocortex. I G Gottlieb (Ed.) *Studies the Development f Beh vior and the Nervo Syst m* Vol. 2 Academic Press, New York 1973 (I press)

Molliver M E. An ontogenetic study of evoked somesthetic cortical responses in the sheep In C. G Bernhard and J P Schadé (Eds.) *Prog ess in B ai Resear h* Vol. 26 *D lopme tal Neurol gy* Elsevier Amsterdam 1967 78—90

Molliver M E. nd H van der Loos, Th ontogenesis of cortical circuity The patial distribution of synapses in somesthetic cortex of newborn dog *Ergebn A t E twickl.-G sch* 1970 *42* 1 -54

Mountcastle V B., Modality nd topographic properties of single neurons of cat s somatic sensory cortex *J N urophysiol* 1957 *20* 408—434

Mountcastle V B. P W D ies and A. L. Berman Response properties of neurons of cat somatic sensory cortex to peripheral stimuli. *J N rophy iol.* 1957 *20* 374—407

Mountcastle V B and T P S Powell Neural mechanisms subserving cutaneous

sensibility with special reference to the role of afferent inhibition in sensory perception and discrimination. *Bull. Johns H pk ns Hosp* 1959 *105* 201—232.

Myslivecek, J The development of the response to light flash in the visual cortex of the dog. *Brain Res.* 1968 a. *10* 418—430

Myslivecek, J Subcortical auditory and visual relays and specific cortical response. In L. Jilek and S Trojan (Eds.) *Ont genesis of the B ain* Charles Univ Prague 1968 b. 359—366.

Noback, C. R. and D P Purpura Postnatal ontogenesis of neurons in cat neocortex. *J omp Neurol.* 1961 *117* 291—307

Nougier M *Etud d s Projections des Lièvres s r le Cortex Cérébral d Me ton p l Techniq d Potentiels Evoqu* Diplome d'études supérieures. Marseille 1963

Perl E. R. and D G Whitlock, Potentials evoked in cerebral somatosensory region. *J Neurophysiol.* 1955 *18* 486—501

Persson, H. E., Electrophysiological studies on the functional development of the somatosensory cortex during early prenatal ontogenesis (In Russian) *X Scientific Conf renc on Ag M phol gy Physiol gy and Biochemistry* Moscow 1971 (In press)

Persson, H. E., Functional development in the somatosensory cortex of foetal sheep. I *J Barcroft Centenary Symposium o Prenatal and N natal Physiology* Cambridge Univ Press, Cambridge 1973 (I press)

Persson, H. E. and D Stenberg, Early prenatal development of cortical surface responses to visual stimuli in sheep. *Exp Neurol.* 1972. *37* 199—208.

Phillis, J W and D H. York, Strychnine block of neural and drug-induced inhibition in the cerebral cortex. *Nat (Lond)* 1967 *216* 922—923

Pittinger C. B and L. E. Morris, Observations of the placental transmission of gallamine triethiodide (Flaxedil®) succinylcholine chloride (Anectine®) nd decamethonium bromide (Syncurine®) in dogs. *Curr R s Ane th* 1955 *34* 107—111

Pollen, D A., Discussion on the generation of neocortical potentials. In H. H. Jasper A. A. Ward and A. Pope (Eds.) *Basic M h isms of th Epilepsies* Little Brown Boston 1969 411—420.

Pollen, D A. and C. Ajmone Marsan, Cortical inhibitory postsynaptic potentials and strychninization. *J Neurophysiol* 1965 *28* 342—358.

Pujol, R., *Maturation postn tale d système auditif h le chat Etud fonctio elle et structurale* Thèse. Montpellier 1971

Pujol R., M. R. Granier and R Marty Maturation post-natale du système aud tif Réponses électro-corticales à la stimulation par sons purs *Rev Neurol.* 1966 *115* 587—590

Pujol, R. and R. Marty Structural and physiological relationships of maturing uditory system. In L. Jilek and S. Trojan (Eds.) *Ont genesis f the Brai* Charles Univ Prague 1968. 377—385.

Purpura, D P Nature of electrocortical potentials and synaptic organizations in cerebral and cerebellar cortex. *Int Rev Neu obiol* 1959 *1* 47—163

Purpura D P Morphological basis of elementary evoked response patterns in the neocortex of the newborn cat. *A N Y Acad. Sci.* 1961 a. *92* 840—859

Purpura D P Analysis of axodendritic organization in immature cerebral cortex. *A N Y Acad. Sci.* 1961 b. *94* 604—654

Purpura, D P Ontogenetic analysis of some evoked synaptic activities in superficial neocortical neuropil. I E. Florey (Ed.) *Nervous I hibition* Pergamon Press, Ox ford 1961 c. 424—446.

Purpura, D. P Synaptic organization of immature cerebral cortex. *Wld Neurol* 1962 *3* 275—298.

Purpura, D P Role of synaptic inhibition in synchronization of thalamocortical ac tiv ty In E. A. Asratyan (Ed.) *Progress in Brain Resear h*, Vol. 22, *Brain Refle es*

Elsevier Amsterdam 1968. 107—122
Purpura, D P Stability and seizure susceptibility of immature brain. In H. H. Jaspers, A. A. Ward and A. Pope (Eds.) *Basic M chanisms of the Epilepsies* Little, Brown, Boston 1969 481—505
Purpura, D P., Synaptogenesis in mammalian cortex. In M. B. Sterman, D J McGinty and A. M. Adinolfi (Eds.) *Brain Development and Behavior* Academic Press, New York 1971 23—41
Purpura, D P Intracellular studies of synaptic organizations in the mammalian brain. In G D Pappas and D P Purpura (Eds.) *Structure a d Functio of Synapses* Raven Press Publishers, New York 1972. 257—302.
Purpura, D P., S. Prelevic and M. Santini, Postsynapti potentials and spike variations in the feline hippocampus during postnatal ontogenesis. *Exp Neurol.* 1968. *22* 408—422.
Purpura, D P R. J Shofer E. M Housepian and C. R. Noback Comparative ontogenesis of structure-f nction relations in cerebral and cerebellar cortex. In D P Purpura and J P Schadé (Eds.) *Prog ess in Brain Research* Vol. 4 *Growth and M t ation of the B ain* Elsevier Amsterdam 1964 187—221
Purpura, D P R. J Shofer and T Scarff, Properties of synaptic activities and spike potentials of neurons in immature neocortex. *J Neurophysiol* 1965 *28* 925—942.
Retzius, G Ueber den Bau der Oberflächenschicht der Grosshirnrinde beim Menschen und bei den Säugethieren. *V erh. biol. V er (Stockh.)* 1891 *3* 90—102.
Retzius, G Die Cajal'schen Zellen der Grosshirnrinde beim Menschen und bei Säugethieren. *Biol. Untersuch (Stockh.)* 1893 *5* 1—8.
Richard P., P Auffray and D Albe-Fessard, Activités thalamiques évoquées par des stimulations somatiques chez le mouton anesthésié au chloralose. *Ele t oen ph clin. Neurophysiol* 1967 *3* 401—409
Romanes, G J The prenatal medullation of the sheep s nervous system. *J Anat (Lond)* 1947 *81* 64—81
Rose, J E., A cytoarchitectural study of the sheep cortex. *J mp Neurol.* 1942. *76* 1—55.
Rose J E. H Adrian and G Santibanez, Electrical signs of maturation in the auditory system of the kitten. *Act neurol. lat -ame* 1957 *3* 133—143.
Rose, G. H., The development of visually evoked electrocortical responses in the rat. *Develop Psy hobiol.* 1968 a. *31* 35—40
Rose G H., The comparative ontogenesis of visually evoked responses in rat and cat. In L. Jílek and S. Trojan (Eds.) *O togenesis f the Brain* Charles Univ Prague 1968 b. 347—358
Rose G H., R lationship of electrophysiological and behavioral indices of visual development in mammals. In M. B. Sterman, D J McGinty and A. M. Adinolfi (Eds.) *B ain Devel pment and Behavior* Academic Press, New York 1971 145—182.
Rose G H., S. P Gruenau and J W Spencer Maturation of visual lectrocortical responses in unanesthetized kittens effects of barbiturate anesthesia. *Electroenc ph. lin N rophysiol.* 1972 *33* 141—158.
Rose G H and D B. Lindsley Development of visually evoked potentials in kittens specific nd nonspecific responses. *J Neurophysiol.* 1968. *31* 607—623
Rubel, E. W A comparison of somatotopic organization in sensory neocortex of new born kittens and adult cats. *J comp Neurol.* 1971 *143* 447—480.
Ruckebusch, Y Activité électro-corticale chez le foetus de la brebis (Ovis aries) t de la vache (Bos taurus) *Rev M d vét* 1971 *34* 483—510.
Schadé J P H. van Backer and E. Colon, Qua titative analysis of neuronal parameters in the maturing cerebral cortex. In D P Purpura and J P Schadé (Eds.) *P gress n Brain Research* Vol. 4 *Growth and Maturation f th B ain* Elsevier Amsterdam 1964 150—175.

Scheibel, A. B., Neural correlates of psychophysiological development in the young organism. In B. Wortis (Ed.) *Recent Advances in Biol gical Psychiatry* Vol. 4 Plenum Press, New York 1962. 313—327
Scheibel, M. E. and A. B. Scheibel Some structural-functional correlates of development in young cats. In W A. Himwich and H. E. Himwich (Eds.) *Progress in Brain Research* Vol. 9 *Th Develop g Brai* Elsevier Amsterdam 1964 6—25
Scheibel, M. E. and A. B. Scheibel, Selected structural-functional correlations in post natal brain. In M. B. Sterman, D J McGinty and A. M Adinolfi (Eds.) *Brain Development and Behavior* Academic Press, New York 1971 1—22.
Scherrer J and D Oeconomos, Réponses corticales somesthésiques d mammifère nouveau-né comparées à celles de l animal adulte. *Etud néonatal.* 1934 3 199—216
Scherrer J R. Verley and L. Garma, Time flow and velocity in early life In L. Jílek and S. Trojan (Eds.) *Ontogenesis of the Brain* Charles Univ Prague 1968. 303—309
Scherrer J R. Verley and L. Garma, A review of French studies in the ontogenetical field. I W A. Himwich (Ed.) *Devel pmental Neurobiology* Charles C. Thomas, Springfield 1970. 528—549
Sedláček, J Cortical responses to visual stimulation in the developing guinea pig during prenatal nd perinatal period. *Physiol. bohemoslov* 1971 20 213—220
Skoglund, S., The spinal transmission of proprioceptive reflexes and the postnatal development of conduction velocity in different hindlimb nerves in the kitten. *Act physiol. scand.* 1960. 49 318—329
Skoglund, S Growth and differentiation with special emphasis on the central nervous system. *Ann. Rev Physiol.* 1969 31 19—42.
Spector W S *Handbook of Biological Data.* W B. Saunders Company Philadelphia and London 1956.
Stefanis, C. and H. H. Jasper Strychnine reversal of inhibitory potentials in pyramidal tract neurones. *Int. J Neuropharmacol.* 1965. 4 125—138.
Thairu, B. K., Post-natal changes in the somaesthetic evoked potentials in the albino rat. *Natur (Lond) New Biol.* 1971 231 30—31
Thatcher R. W and D P Purpura Maturational status of inhibitory and excitatory synaptic activities of thalamic neurons in neonatal kitten. *Brain Res* 1972. 44 661—665.
Thompson, R. F L. A. Bettinger H Birch and P M. Groves, Comparison of evoked gross and unit responses in association cortex of waking cat. *Electroenc ph clin Neurophysiol.* 1969 27 146—151
Towe, A. L., On the nature of the primary evoked response. *Exp Neu ol.* 1966. 15 113—139
Towe, A. L. and G W Harding, Extracellular microelectrode sampling bias. *Exp Neurol.* 1970 29 366—381
Towe, A. L. and T T Kennedy Response of cortical neurons to variation of stimulus intensity and locus. *Exp Neurol.* 1961 3 570—587
Verley R., Recherches sur le développement des activités électro-corticales avec des électrodes corticales radiaires. *J Physiol. (Paris)* 1965. 57 407—436.
Verley R., Etude comparée de l'évolution des vitesses de conduction centrales et périphériques cours de l'ontogénèse. *J Physiol. (Paris)* 1967 a. 59 306—307
Verley R., Relations de noyaux d thalamus dorsal avec le cortex cérébral t avec la périphérie au cours d l'ontogénèse. *J Physiol. (Paris)* 1967 b. 59 525.
Verley R., Etude stratigraphique des activités électrocorticales provoquées dans le cortex cérébral du lapin nouveau-né. *J Physiol. (Paris)* 1968. 60 321
Verley R. and R. Rokyta Ontogenèse d potentiel évoqué cortical somesthésique chez le lapin nouveau-né. *Arch. sci. physiol.* 1972. 26 267—276.
Verley R. and G Siou Relations spatiales de quelques structures diencéphaliques et

mésencéphaliques chez le lapi nouveau-né. *J Physiol. (P ris)* 1967 *59* 257—279
Verley R., G Siou and L. Garma, Potentiels corticaux provoqués par stimulation thalamique chez le lapi nouveau-né. *J Phys ol (P ris)* 1966. *58* 272—273
Vernandakis, A. and D M. Woodbury Electrolyte and amino acid changes in rat brain during maturation. *Amer J Physiol* 1962 *203* 748—752.
Wiesel T N and D H. H bel, Single-cell responses in striate cortex of kittens deprived of vision in one eye *J Neurophysiol.* 1963 *26* 1003—1017
Vignal, W., Recherches sur le développement des éléments des couches corticales du cerveau et d cervelet chez l'homme et les mammifères. *Arch physiol. norm pathol.* 1888 2 228—254
Windle, W F *Physiology f the Fetus.* W B. Saunders Company Philadelphia and London 1941
Voeller K. G. D Pappas and D P Purpura, Electron microscope study of development of cat superficial neocortex. *Exp Neurol* 1963 *7* 107—130.
Woolsey C. N and D Fairma Contralateral, ipsilateral, and bilateral representation of cutaneous receptors in somatic areas I and II of the cerebral cortex of pig, heep and other mammals. *S rgery* 1946 *19* 684—702.

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 395

From the Department of Medical Pharmacology
University of Uppsala, Sweden

Monoaminergic Influence on Testosterone-Activated Copulatory Behavior in the Castrated Male Rat

By

Carl Olof Malmnäs

I Testosterone-activated copulatory behavior in the castrated male rat.
II. Monoamine precursors and copulatory behavior in the male rat.
III. Copulatory behavior in the male rat after impaired monoaminergic neurotransmission.
IV Effects of LSD-25 clonidine and apomorphine on copulatory behavior in the male rat.

UPPSALA 1973

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 395

From the Department of Medical Pharmacology
University of Uppsala, Sweden

Monoaminergic Influence on Testosterone-Activated Copulatory Behavior in the Castrated Male Rat

By
Carl Olof Malmnäs

I. Testosterone-activated copulatory behavior in the castrated male rat
II. Monoamine precursors and copulatory behavior in the male rat.
III. Copulatory behavior in the male rat after impaired monoaminergic neurotransmission.
IV Effects of LSD-25 clonidine and apomorphine on copulatory behavior in the male rat.

UPPSALA 1973

Co-existence or no existence
that is the question : if no copulation
no population

(Partly from Piet Hein)

To my father

CONTENTS

PAPER I

TESTOSTERONE-ACTIVATED COPULATORY BEHAVIOP IN [illegible]

MALE RAT

INTRODUCTION

MATERIAL AND GENERAL METHODS

Subjects 10; Testing procedure 11; Observat[illegible]
The experimental d sign: A. Chronological sc[illegible]
treatment 13; B Statistical methods 13; C Pre-experi-
mental stat s of subjects 13; Injected materia[illegible]

STUDIES ON THE EXPERIMENTAL PROCEDURE

A Copulatory behavio in the intact male ra[illegible]
 1 Di tribution of mount and ejaculation [illegible]
 2 The number f mounts ach min during [illegible]
 10 min 15;
B The effect of castration
 1 Expl ratory behavior 16;
 2 Copulatory parameters 17;
C The effect of a single inj ction of tes[illegible]
 pi nate (TP)
 1 The latency to nset of the hormone ef[illegible]
 2 The duration f the ffect on mount [illegible]
 singl dos of TP 21;
 3 The eff ct of single doses of TP [illegible]
 meters 22;
D The effect of weekly TP injections
 1 Day of maximal response 23;
 2 Dose-respons relationship 24;
 3 The long-term effect of TP given [illegible]
 4 Di tribution of the copulatory para[illegible]
 kept on weekly TP treatment 26;
 5 Distribution of jaculation laten[illegible]
 kept on submaximal TP treatme[illegible]
 6 The acute effects of fr e testos[illegible]
 7 The effect f increasing the [illegible]
 8 The effect on mount percentage [illegible]
 t atment 31;
E The influence f the adrenals
 1 The copulat ry response one we[illegible]
 2 Th effect of long-acting AC[illegible]
 3 The effect f short-acting [illegible]
 mized and adrenalectomized [illegible]

F Studies including other behavior patterns than copulatory 37
1 A simple way t score the orientation of activity during copulatory tests 37;

DISCUSSION 40

CONCLUDING REMARKS 44

REFERENCES 45

PAPER II

MONOAMINE PRECURSORS AND COPULATORY BEHAVIOR IN THE MALE RAT

ABSTRACT 47

INTRODUCTION 47

MATERIAL AND METHODS 48

Experimental subjects 48 Injected materials 49 Testing procedure 49; Copulatory parameters recorded 49; Experimental design of copulatory tests 50; Methods to evaluate the specificity of drug induced changes of copulatory behavior: A Motor activity 50; B Scoring of the orientation of activity during copulatory tests 51; Statistical analysis 51,

RESULTS 52

The eff ct of monoamine oxidase inhibitors 52;
The effect of a subeffective dose of pargyline in combination with monoamine precursor amino acids 52;
The effect of monoamine precursor amino acids after extracerebral decarboxylase inhibition 57;
The effect of DL-DOPS 58;
Motor activity 58;
Scoring of the orientation of activity during copulatory tests 61;

DISCUSSION 64

REFERENCES 67

PAPER III

COPULATORY BEHAVIOP IN THE MALE RAT AFTER IMPAIRED MONOAMINERGIC NEUROTRANSMISSION

ABSTRACT 69

INTRODUCTION 69

MATERIAL AND METHODS 70

Subjects 70; Inj t d material 71

RESULTS 71

The effect of reserpine 71;
The effect of tetrabenazine 73;
The effect of p-chlorophenylalanine (PCPA) 73;
The effect of PCPA in adrenalectomized subjects 75
The effect of α-methyl-p-tyrosine (αMT) 75;
The effect of FLA-63 78;
The effect of chlorpromazine 79;
The effect of pimozide 80;
The eff ct of phenoxybenzamine 81;
The eff ct of propranolol 82
Motor activity 84;
Scoring of the rientation of activity during copulatory tests 85;

DISCUSSION 91

REFERENCES 94

PAPER IV

EFFECTS OF LSD-25 CLONIDINE AND APOMORPHINE ON COPULATORY BEHAVIOR IN THE MALE RAT

ABSTRACT 96

INTRODUCTION 96

MATERIAL AND METHODS 97

Subjects 97; Inj cted materials 97;

RESULTS 98

The effect of LSD 98;
The effect f lonidin 98;
The effect f apomorphine 103;
The eff ct f apomorphine after pimo id pretreatment 103;
Motor activity 104;
Sc ring f the orientation of activity during copulat ry tests 104;

DISCUSSION 111

REFERENCES 115

MONOAMINERGIC INFLUENCE ON TESTOSTERONE-ACTIVATED COPULATORY BEHAVIOR IN THE CASTRATED MALE RAT

GENERAL SUMMARY AND CONCLUSIONS 117

THE METHOD 117

NEUROPHARMACOLOGICAL EXPERIMENTS 119

Increase of the monoaminergic tone 119;
Decrease of the monoaminergic tone 120;
The effect of monoaminergic agonists 122;

CONCLUSIONS 122

Mount percentage 123;
Other copulatory parameters 123
Orientation of activity 124;
Copulatory behavior in the male and female 124;

ACKNOWLEDGEMENTS 126

REFERENCES 127

The papers I II III and IV will in the papers be referred to as Malmnäs 1973a 1973b 1973c and 1973d respectively

From the Department of Medical Pharmacology
University of Uppsala Uppsala Sweden

Testosterone-Activated Copulatory Behavior in the Castrated Male Rat

By

CARL OLOF MALMNÄS

INTRODUCTION

The predominant behavior pattern which is displayed by the male rat during heterosexual intercourse is a repetitive mounting of the female partner. On mounting the male clasps the flanks of the female and exerts pelvic thrusts. When mounted by a male the receptive female raises her perineal region which permits the male to achieve intromission of the penis into the vagina. Although this posture is always displayed by the receptive female when mounted every mount does not result in intromission. When intromission occurs a vigorous backward lunge is shown by the male when he dismounts. Dismounts after intromission are followed by genital licking while this behavior is less regular and of shorter duration when intromission has not been achieved. After a certain number of intromissions (5-15) the ejaculatory reflex is elicited recognized by a prolonged mount with a vigorous clasp around the female followed by a slow dismount and subsequent extended genital licking. After ejaculation there is a certain time interval (refractory period) until the male starts to mount the female again.

Copulatory behavior in the male rat is dependent on gonadal hormones. After castration there is a gradual decline in copulato-

ry performance which can be restored to the pre-castration level by testosterone treatment (Shapiro 1937; Stone 1939; Beach and Holz-Tucker 1949) The propionic acid ester of testosterone (TP) is most commonly used in this context.

Previously neuropharmacological studies on heterosexual copulatory behavior in the male rat have exclusively been conducted in the intact subject (Zimbardo and Barry 1958; Gillett 1960; Soulairac 1963; Bignami 1966; Butcher et al 1969; Leavitt 1969; Dewsbury and Davis 1970; Whalen and Luttge 1970; Ahlenius et al 1971; Hyyppä et al 1971; Salis and Dewsbury 1971; Tagliamonte et al 1971; Dewsbury 1972; Dewsbury et al 1972) The testing procedure which has been used in these studies usually allows the subject to copulate until the occurrence of one or more ejaculations Interest has been focused on effects of drugs on parameters of the established copulatory behavior and the post-ejaculation refractory period in as a rule vigorously copulating subjects However it is an advantage to work with castrated animals supplied with exogenous hormone in neuropharmacological studies on this behavior: 1 drug effects on the gonadal hormone production are avoided and 2 it is easier to maintain a submaximal response level in order to allow a stimulatory or inhibitory effect of a drug treatment to be seen

This investigation deals mainly with pre-ejaculation copulatory (= mounting) behavior in the adult castrated male rat The main aim was to find a schedule of TP treatment that induced and could maintain a submaximal response with respect to the percentage of subjects that displayed mounting behavior

MATERIAL AND GENERAL METHODS

Subjects

Experimental subjects About 450 male Wistar rats weighing 350-450 grams when u d in xperiments (purchased as Specific Pathogen Free Möllegaa d Ejby, Denmark) The animals were housed thr e in each cage (Ma rolon[R] 34x40x15 cm) in a room with forced venti-

lation at 21 ± 1°C under a reversed day-night light cycle (light from 10 pm to 11 am) Commercial rat pellets (Anticimex 210 Sollentuna Sweden) and tap water were given ad libitum

Stimulus females Ovariectomized Sprague-Dawley female rats were kept in the laborat ry under the same conditions as the experimental subjects The females were brought into artificial estrous by 25 µg estradiol benzoate followed 48 hours later by progesterone 1 mg s c and were used as stimulus objects 4-7 hours after the progesterone injection By this hormone treatment the females displayed an intens estrous behavior with darting movements and presentations to the male Receptivity on mounting by intact male rats was ch cked before the xperiments Only highly receptive females were used

Testing Procedure

Copulatory tests were performed during the dark period of the light cycle (between 2 pm and 5 pm) under dimmed light conditions The male was transferred from the home cage to an obs rvation cage (se below) and a stimulus female was brought into the observation age 5 min later (se fig 1) The time allowed from the introduction of the female to the occurrence of the first mount was restricted to 3 min If the male did not mount the female the test was end d after 3 min If the male mounted the female within 3 min from her introduction the test continued for another 3 min counted from the first mount or until ejaculation occurred whichever came first The records taken were based on the perfor-



Fig 1 Diagrammatic representation of testing the copulatory behavior in male rats
A, Duration of the tanda d test if no mount oc urs
B Mount l t ncy
C Duration of the tanda d test if at least one mount occurs
Note C is of the same length as A
D Intromission latency
E Ejaculation latency

nance during these 3 min The observation cages were arranged so that 3 subjects could be tested simultaneously From the records taken the following measures could be determined:

1 Mount percentage
The p rcentage of subjects which displayed at least one mount with or without intromission during the test; only mounts with evident pelvic thrusts were recorded
2 Mount latency*
The time (expressed as hundredths of a min) from the introduction of the female to the first mount with or without intromission (see fig 1:B)
3 Number of mounts per min.*
Total number of mounts with or without intromission during the test divided by the length in min of the complete 3 min test i e total number of mounts/3 Thus subjects which ejaculated before the 3 min period was ended were excluded from this calculation
4 Intromission percentage
The percentage of subjects which displayed intromission within 3 min from the introduction of the female
5 Intromission latency*
The time (expressed as hundr dths of a min) from the introduction of the female to the occurrence of the first intromission if this occurred within 3 min from the introduction of the female
6 Intromi sion ratio
The percentage of mounts with intromission of the total number of mounts Only subjects which displayed intromission within 3 min from the introduction of the female were included
7 Ejaculation p rcentage
The percentage of subjects that displayed ejaculation within 3 min from the first mount
8 Ejaculation latency*
The time from the first intromission to ejaculation (fig 1:E)

Note: Parameters 2 3 5 6 and 8 were recorded for each individual subject that displayed the behavior in question while parameters 1 4 and 7 are perc ntage numbers based on several subjects

Observation cages

Tests were performed in rectangular wooden cages (40x60x40 cm) with a 27 cm high plexiglass front and a wire mesh top On the Masonite® floor there were wooden shavings Animals were brought into the cage through the 13 cm wid opening between the plexiglass front and the top cover

*Footnote: Times were measured by means of a stop-watch the scale of which was divided into hundredths of a min

The Experimental Design

A Chronological schedule of treatment

1 Pre-castration tests: The experimental subjects were given opportunity for sexual experi nce They were tested with sexually receptive females for 20 min twice weekly This regimen was con tinued until the first ejaculation was observed 2 Castration: Within a few days after the first recorded ejaculation the subjects were castrated by scrotal incision under ether anesthesia At th time of castration they were about 100 days of age 3 Post-castration tests: a) After castration the subjects were tested for copulat ry activity once weekly From this point on the subjects were *tested a cording to the testing procedure stated above* b) Wh n in two successive post-castration tests the mount percentage of th batch was less than 50% the animals were given TP once weekly The copulatory tests were performed 3 or 4 days after the TP injection

B Statistical methods

1 Copulatory beh vior: A pre-experimental test means the last test preceding a treatment and an experimental test means the test following a treatment Blank treated subjects were tested parall l to th experimental subj cts The pre-experimental test was used as comparison when a stati tical ignificance of the effe t of an experiment l treatment was calculat d Thus the subjects served as their own controls Non-parametric statistical tests were used (Siegel 1956) namely the sign test (to analyze the significance of diff rences in mount percentage intromission percentage and ej culation percentage) and the Wilcoxon matched-pairs signed-anks te t (to analyze the significance of differences in mount lat ncy number of mounts/min intromission ratio and the number f mounts ach min in exp A:2) 2 For the analysis of differences in mot r a tivity between experimental and control subjects the Mann-Whitney U test was used

The 5 per cent level of significance was used (two-tailed probabilities)

C Pre-experimental states of subjects

I Sexually xperi nced intact subj cts

IIa Castrated subj cts (never given TP) drawn from a batch of animals with a mount percentage less than 50%

IIb Castrated subje t *which had been subjected t a* submaximal TP treatment but not given TP during th last 3 w eks prior t the experiment

III Castrat d subj cts which were tested once weekly and maintained on submaximal TP treatment given 3 or 4 days prior to ach weekly test

Since ther was no *ignificant diff rence* in respons between subjects in pre-exp imental t t IIa and IIb in xp C1 and C3

the data were pooled

Injected Materials

The hormones used were testosterone propionate (TP) free testosterone estradiol benzoate and progesterone (Organon) dissolved in olive oil free dexamethasone (Organon) suspended in olive oil and thoroughly shaken before injection and porcine ACTH [short acting (amorphous) Acton[R] Ferring and long acting (zinc chloride prolongated release) Cortrophin[R] prol Organon through Pharmacia] diluted in 0 9% saline All injections were given subcutaneously in a volume f 1 ml/kg body weight

STUDIES ON THE EXPERIMENTAL PROCEDURE

A COPULATORY BEHAVIOR IN THE INTACT MALE RAT

Since the main interest in this investigation was focused on pre-ejaculation copulatory behavior the design of the testing procedure was made with regard to this aim. The duration of a test should be long enough to allow mount latency and the number of mounts/min to be determined The length of time a test should continue in order to meet these requirements was tested in exp A. (See also exp D4 and D5 for TP treated castrated males)

Experiment A 1 Distribution of Mount and Ejaculation Latencies

Procedure

30 subjects in pre-experimental state I (see Methods) were allowed to copulate until they achieved ejaculation

Results

The distribution of mount latencies is given in fig 2 The majority of subjects mounted the female within half a min. after the female was introduced (median 10 min /100) and all subjects had a

Fig 2 Mount latencies Distribution in sexually experienced intact male rats (N = 30)

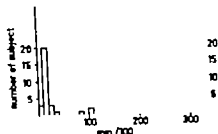


Fig 3 Ejaculation latencies Distribution in sexually xperienced intact mal rats (N = 30)

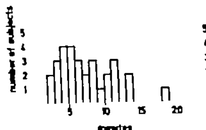


Fig 4 The number of mounts ach min (mean _ s e m) during the first 10 min counted from the first mount Sexually experienced intact male rats with ejaculation latencies gr t r than 10 min. (N = 18)



mount latency shorter than 2 min. The distribution of ejaculation latencies is given in fig 3 No subject ejaculated within the first or second min. after the first intromission while during the third min., two subjects ejaculated. The median ejaculation latency was 6 3 min All subjects ejaculated within 20 min. from the first intromission.

Experiment A 2 The Number of Mounts Each Min during the First 10 Min

P ocedure

From a batch of subjects in pre-experimental state I (see Methods)

18 males with an ejaculation latency in an earlier run of between 10 and 20 min. were selected The number of mounts each min for 10 consecutive min. counted from the first mount was determined.

Results

The distribution of the average number of mounts each min. during the first 10 min. is given in fig 4 During the first 4 min there was a gradual decline in the frequency of mounts from 5 3 the first min to 3 6 the fourth min. ($p < 0\ 01$) After 4 min no further significant decrease was seen

Conclusion from exp A 1 and A 2 The data show that 3 min. of testing is enough to cover mount latency Counted from the first mount 3 min is also a suitable time for the number of mounts/min to be determined since very few subjects ejaculated within this time

B THE EFFECT OF CASTRATION

Experiment B 1 Exploratory Behavior

Procedure

6 castrated and 6 sham-castrated subjects were used 5 months after surgery One male at a time was run for 10 min in an Animex[R] activity meter (Svensson and Thieme 1969) (movements of an animal across a tuned oscillator coil system results in a change of the tuning which is recorded as a count) The kind of motor activity displayed during the 10 min period was mainly exploring of the cage The experiment was performed during the dark period of the day

Results

There was no significant difference in the number of counts scored by subjects in the two treatment categories (table I) Thus this experiment shows that the type of motor activity which is measured

TABLE I The effect of castration on motor activity in male rats. Surgery was done 5 months prior to the measurement. One animal at a time was run for 10 min. in an Animex activity meter

MOTOR ACTIVITY

Treatment	Count /10 min ± m	P	N
Sham-castrati	923 ± 41	0 05	6
Castr tion	963 64		6

by this method is not decreased 5 months after castration.

Experiment B 2 Copulatory Parameters

Procedure

65 subjects in pre-experimental state I (see Methods) were subjected to a 3 min. standard copulatory test (pre-exp test) One day after this test 52 of the subjects were castrated and 13 were sham-operated. Tests for copulatory behavior were conducted once weekly starting one week after surgery

Results

The results are given in fig 5 and table II All sham-castrated subjects mounted and had intromissions throughout the 7 week experimental period Compared to the pre-exp test no significant changes of the parameters occurred except for the intromission latency which was significantly reduced. In contrast the castrated subjects showed a continuous reduction in mount percentage and intromission percentage Five weeks after castration 50% of the castrated males mounted the female. The number of mounts/min. gradually declined and the mount latency and intromission latency became continuously longer after castration The intromission ratio however remained unchanged. The intromission latency was significantly increased at 1 through 6 weeks after castration but not at 7 weeks The data obtained at 7 weeks were influenced by a

TABLE II The effect of castration on copulatory behavior in male rats The pre-experimental test was performed one day before surgery and the experimental test at 7 weeks post-castration. Md = median, a = p 0 05 b = p 0 01 c = p 0 001

Copul tory parameter	Sham-castratio P e-exp	Exp	N	Ca tration Pre-exp	Exp	N
Mount						
Percentage	100	100	13	100	44[c]	52
Latency[Md]	10	10	13	10	40[b]	23
No/min[Md]	3 3	3 3	12	4 0	2 0[b]	21
Intromi sio						
Percentage	100	100	13	100	23[c]	52
Latency[Md]	30	15	13	15	40	12
Ratio[Md]	63	63	13	60	69	12
Ej cul ti n						
Percentage	0	8	13	6	0	52

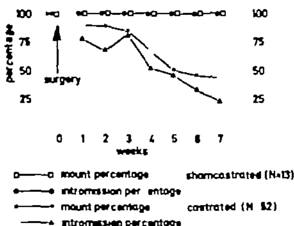


Fig 5 The effect of castration and sham-cast ation on mount percentage and intromission perc ntage in the male rat

relatively great reduction in one subject and by the smaller number of males which displayed intromission

At the beginning of each test all subjects approached the female irrespective of subsequent mounting or non mounting If they did not mount the female a major part of the first min was devoted to anogenital sniffing of and other social interaction with her Regarding the exploratory activity oriented towards the environment there was no obvious decrease after castration.

C THE EFFECT OF A SINGLE INJECTION OF TESTOSTERONE PROPIONATE (TP)

The following experiments (C 1-3) were carried out in order to study the effect of different doses of TP on copulatory behavior in the castrated male rat with regard to latency to onset duration and magnitude of response

Experiment C 1 The Latency to Onset of the Hormone Effect

Procedure

Out of 212 subjects in pre-experimental states IIa and IIb 120 did not mount in the first test (= Day -1) These 120 subjects were then tested once daily for another 4 consecutive days Six hours before the second test (= Day 0) a single injection of TP free testosterone (T) or oil vehicle was given as shown in fig 6

Results

Fig 6 shows the distribution in time of the appearance of the first mount after the different treatments given The number of animals mounting after TP treatment at 6 and 30 hours after the injection was not significantly different from the oil blank results A consistent effect in all TP treated groups was that the distribution reached its highest value 54 hours after the TP injection. This peak was not seen in the oil treated controls A rough estimate of the median latency for TP to activate mounting

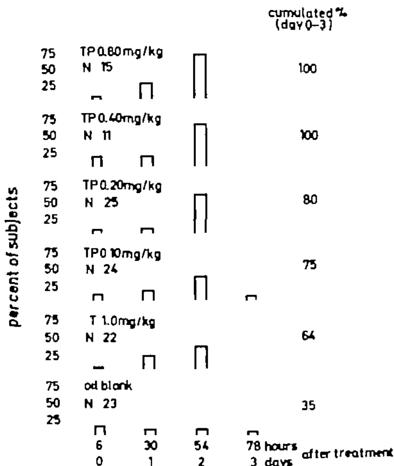


Fig 6 Lat ncy to onset of the hormone effect in non-mounting subjects Distribution of the time interval from the administration of TP or T (free testost rone) to the appearance of the first mount The injecti ns were given 6 hours before the test at Day 0 and th subj cts were tested once daily for 4 consecutive days until the appearanc of the first mount Castrated male rats in pre-experimental stat IIa and IIb (N = 120)

in the subjects is about 30-54 hours Essentially the same results were obtained after treatment with free testosterone 1 0 mg/kg

Experiment C 2 The Duration of the Effect on Mount Percentage of a Single Dose of TP

Procedure

61 subjects in pre-experimental state IIa were used. They were divided into 4 groups which received either TP 0 10 (N = 15) 0 20 (N = 15) or 0 40 (N = 21) mg/kg or oil blank solution (N = 10) The TP treatment was given 6 hours before the test at Day 0 Tests for copulatory behavior were conducted at Day -1 0 1 2 3 5 10 15 and 20

Results

Mount percentages for the different days after treatment are given in fig 7 At the day before treatment the response rate was about 40-50 % for the different treatment categories No effect of the testosterone treatment was seen at Day 0 and 1 An evident in-

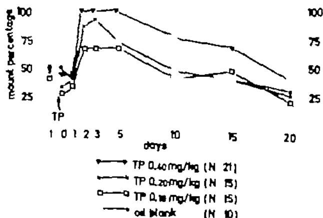


Fig 7 The dur tion f the effect of a ingle dose of TP on mount p centage in castrat d male rats (N = 61) Th treatments were given 6 hours before the test at Day 0

crease in response was obtained at Day 2 and this response was maintained in all treatment categories at Day 3 At Day 10 there was still an evident effect after TP 0 40 mg/kg but not after the lower doses

Experiment C 3 The Effect of Single Doses of TP on Copulatory Parameters

Procedure

126 subjects in pre-experimental states IIa and IIb were used They were divided into 3 groups These groups were given either oil vehicle (N = 40), TP 0 10 mg/kg (N = 65) or TP 0 40 mg/kg (N = 21) and were tested for copulatory behavior 3 days after the injections

Results

Both TP doses used induced a significant increase in mount percentage while no significant effect on this parameter was seen in the oil treated controls (table III) The intromission percentage and the number of mounts/min increased significantly after TP 0 40 mg/kg but no significant effect was obtained after TP 0 10 mg/kg A significant reduction in mount latency was obtained already after TP 0 10 mg/kg and also after TP 0 40 mg/kg In the oil treated controls the number of mounts/min. decreased between the pre-experimental and experimental test situation but in other respects there were no significant changes between the two tests

D THE EFFECT OF WEEKLY TP INJECTIONS

In this section (exp D 1-8) different aspects of copulatory behavior were studied using single TP doses given repeatedly once weekly

TABLE III. The effect of single doses of testosterone propionate (TP) on copulatory behavior in castrated male rats. TP was given 3 days before the experimental test which was conducted one week after the pre-experimental test. Md median, a = p 0 05 b = p 0 01, c = p 0 001

Copulatory parameter	Treatment mg/kg Oil blank 0 4 ml Pre-exp	Exp	N	TP 0 10 Pre-exp	Exp	N	TP 0 40 Pre-exp	Exp	N
Mount									
Percentage	50	45	40	42	62[b]	65	43	100[c]	21
Latency Md	63	53	14	60	15[a]	26	40	15	9
No/min Md	2 7	2 0[b]	14	2 7	2 3	26	2 0	3 3	9
Intromission									
Percentage	38	38	40	28	38	65	29	90[c]	21
Latency Md	53	45	8	70	40	13	38	18	6
Ratio Md	75	60	8	50	40	13	64	46	6
Ejaculation									
Percentage	0	0	40	0	0	65	0	0	21

Experiment D 1 Day of Maximal Response

Procedure

28 subjects in pre-experimental state III were used. The subjects were divided into 3 groups and were given TP 0 14 mg/kg once weekly for 3 consecutive weeks At the first injection of the present experiment 2 weeks had elapsed since the last TP injection Each group was subjected to a different day of testing every week either 1 3 or 5 days after the TP injection

Results

The results obtained at the different days of testing after TP injections are given in table IV Only very small differences in the recorded parameters were found between the various days of testing The only significant one was a longer mount latency at day 1 than at day 3 and day 5 Although there were very small dif-

TABLE IV The copulatory behavior at Day 1 3 and 5 after a weekly injection of testosterone propionate (TP) 0 14 mg/kg in castrated male rats Md = median a = p < 0 05

Copulatory parameter	Day after TP treatment Day 1 p (Day 1 3)	N	Day 3 p (Day 3 5)	N	Day 5 p (Day 1 5)	N
Mount						
Percentage	54	28	57	28	50	28
Latency[Md]	70[a]	13	30	13	30[a]	12
No/min[Md]	2 7	13	2 3	12	2 5	12
Intromissio						
Percentage	36	28	36	28	36	28
Latency[Md]	47	6	40	6	30	6
Ratio[Md]	50	6	72	6	54	6
Ejaculation						
Percentage	0	28	0	28	4	28

ferences between the different days of testing it was considered an advantage to have a standard time between the TP injection and the copulatory tests In all subsequent experiments copulatory tests were performed 3 or 4 days after the TP injection

Experiment D 2 Dose Response Relationship

Proc dur

52 subjects in pre-experimental state IIa were used. They were divided into 4 groups and were subjected to 5 weekly tests 3 or 4 days after TP administration Four treatment categories were used: oil blank solution TP 0 050 mg/kg TP 0 10 mg/kg and TP 0 20 mg/kg Each group was run at one dose level only

Results

In the pre-exp tests (fig 8 week 0) the mount percentage was

about 20-25 % for all groups The mount percentage of the group which received TP 0 20 mg/kg once weekly increased to 80 % and remained on this level from week to week The response after TP 0 10 and 0 050 mg/kg stabilized at 40 % and 33 % respectively The oil treated controls decreased slightly in response level from 20 % to 10 % after 4 weeks It is evident from this experiment that a weekly maintainance dose of TP 0 20 mg/kg or less will give a submaximal response for at least 5 weeks It is also evident that the response (mount percentage) is dose-dependent.

Experiment D 3 The Long Term Effect of TP Given Once a Week

Procedure

24 subjects in pre-experimental state IIa were used They were given TP 0 14 mg/kg/week for 20 consecutive weeks Copulatory tests were performed 3 or 4 days after TP injections

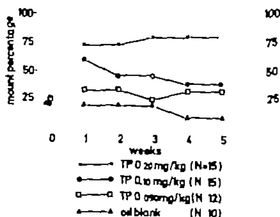


Fig 8 Dose - respons relati nship f weekly administered dos s f TP n mount per entage in castr ted mal rats (N = 52) Cop latory te t were perf rmed 3 4 day after the weekly TP tr atment which tarted at week 1

Results

The mount percentage during the entire test period is given in fig 9 The maximal response 67 % was obtained in the first and second week and the lowest response 41 % was obtained at week 6 Throughout the testing period the response was within this range There was no tendency towards a systematic shift of the response with time

Experiment D 4 Distribution of the Copulatory Parameters in Subjects Kept on Weekly TP Treatment

Procedure

156 subjects in pre-experimental state III were used and the TP dosage was 0 14 mg/kg/week The subjects had been maintained on this schedule of TP treatment for 4 months The testing procedure in this experiment was different in one respect from the one stated in Methods: subjects which had not performed any intromission during the ordinary 3 min of testing were allowed another 10 min of heterosexual contact in the observation cage

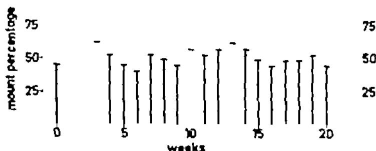


Fig 9 The long term effects on mount percentage of a weekly administered dose of TP The TP dose used was 0 14 mg/kg/week given 3 or 4 days befor the copulatory tests starting at week 1 Castrated male rats (N = 24)

Distribution of the Copulatory Parameters in Subjects Kept on Weekly TP Treatment (0 14 mg/kg)

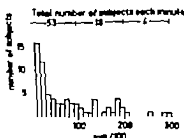


Fig 10 Mount laten ies Distribution in TP tre ted castrated male rats (N = 75)

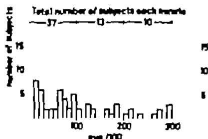


Fig 11 Intromission latencie Distribution in TP treated castrated male rats (N = 60)

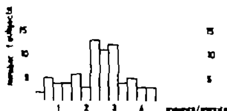


Fig 12 Number f mounts/min Distribution in TP treated castrated male rats (N = 75)

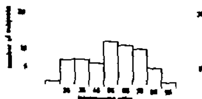


Fig 13 Intromission ratios Di tribution in TP treated castrated mal rats (N = 60)

Results

A. Ordinary testing procedure: No subject ejaculated during the test The mount percentage was 48 % and the intromission percentage was 38 % As shown in fig 10 and 11 the majority of subjects had mount and intromission latencies shorter than 1 min (medians 40 and 85 min /100 resp) The distribution of mounts/min is given in fig 12 The median value is 2 7 and there is a fairly symmetrical distribution around this value The distribution of intromission ratios is given in fig 13 Also this distribution is fairly symmetrical around a median value of 55

B Extended time of testing: Of 81 subjects which did not mount during the ordinary 3 min. only 3 subjects mounted when allowed another 10 min. of testing Of the 15 subjects which mounted without intromission during the ordinary 3 min 4 subjects had at least one intromission during the additional 10 min

Experiment D 5 Distribution of Ejaculation Latencies in Subjects Kept on a Submaximal TP Treatment for Several Months

Procedure

23 subjects in pre-experimental state III were used and the TP dosage was 0 15 mg/kg/week The subjects had been maintained on this schedule of testing and TP treatment for 8-11 months Subjects which performed at least 3 intromissions during a standard test (3 min.) were allowed to continue copulation until the occurrence of ejaculation

Results

The distribution of ejaculation latencies is given in fig 14 No subject ejaculated within 3 min from the first achieved intromission. Most subjects had ejaculation latencies shorter than 10 min. with a median of 8 9 min. All subjects ejaculated within 30 min from the first intromission

Experiments D 6 The Acute Effects of Free Testosterone

Procedure

57 subjects in pre-experimental state III were used. They had been maintained on a TP dosage of 0 15 mg/kg/week for 8 months This dose of TP was also given 3 days before the experimental test of the present experiment. One half of the subjects was given free testosterone 1 0 mg/kg one hour before the experimental test and the other half had olive oil 1 0 ml/kg given at the same time before test Four hours later all subjects were retested. Two weeks after these tests the experiment was repeated but at this time the treatments were reversed; i e subjects which had received free testosterone at the first test were now given oil and vice versa In the statistical analysis of this experiment the test values obtained after oil treatment were compared to the test values obtained after treatment with free testosterone

Results

The results are given in table V Compared to oil treatment no statistically significant effect was found after free testosterone 1 0 mg/kg neither at 1 nor at 5 hours after its injection It can thus be concluded that an addition of a comparatively high dose of free testosterone has little or no effect within five hours from its administration on the copulatory parameters recorded.

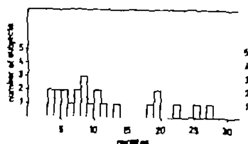


Fig 14 Ejaculation l t nci s Di tributi n in TP treated castrat d mal rats (N 23)

TABLE V The effect of free testosterone (T) on copulatory behavior in castrated male rats maintained on a TP treatment of 0.15 mg/kg/week. Copulatory tests at zero hour Md = median.

Copulatory parameter	Treatment mg/kg					
	Free testosterone 1 0 at -1 hrs			Free testosterone 1 0 at -5 hrs		
	Oil	T	N	Oil	T	N
Mount						
Percentage	56	53	57	47	46	57
Latency^Md	15	15	25	10	10	17
No/min^Md	2 0	2 3	25	2 3	2 3	17
Intromission						
Percentage	39	35	57	25	21	57
Latency^Md	23	20	14	15	20	7
Ratio^Md	60	50	14	57	23	7
Ejaculation						
Percentage	2	2	57	2	2	57

Experiment D 7 The Effect of Increasing the Dose of TP

Procedure

84 subjects in pre-experimental state III were used They had been maintained on a weekly TP dose of 0 15 mg/kg for 9 months prior to this experiment. In this experiment 44 of the subjects received TP 0 20 mg/kg 3 days before testing instead of the usual dose of 0 15 mg/kg while the other 40 subjects were given the same dose of TP that they had been given previously

Results

As is shown in table VI there was a significantly increased mount percentage and a decreased mount latency by an increased dose of TP while other parameters were not significantly changed This experiment shows that the subjects still after several months of a submaximal TP treatment have the capacity to display an increased response to an increase of the TP dose

TABLE VI The effect on copulatory behavior of increasing the dose of testosterone propionate (TP) in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly TP injections Md = median.
a = p 0 05

Copulatory parameter	TP treatment mg/kg 0 15 Pre-exp	0 15 Exp	N	0 15 Pre-exp	0 20 Exp	N
Mount						
Percentage	43	38	42	44	62[a]	45
Latency[Md]	15	15	13	15	10	17
No/min[Md]	2 7	3 0	13	2 5	3 0	16
Intromis ion						
Percentage	31	36	42	29	38	45
Latency[Md]	43	43	8	25	25	11
Ratio[Md]	59	53	8	57	71	11
Ejaculation						
Percentage	0	0	42	2	0	45

Experiment D 8 The Effect on Mount Percentage of Discontinued TP Treatment

Procedure

52 subjects in pre-experimental state III were used They had been kept on a weekly TP treatment of 0 14 mg/kg for 5 months prior to this test and were on a stable response level In this experiment 28 of the subjects were given oil vehicle instead of TP one week and the other 24 subjects had their usual TP treatment (test 2) One week after test 2 all 52 subjects were given TP 0 14 mg/kg (test 3)

Results

The effect of discontinued TP treatment on mount percentage is shown in fig 15 In subjects which were maintained on a weekly TP treatment the mount percentage was kept at roughly the same

level in test 1 through 3 Discontinued TP treatment (test 2) brought about a significant decrease in mount percentage from 54% to 14% ($p < 0.001$ sign test) The response was restored to almost the same level as in test 1 when TP was given 2 weeks after the last TP treatment (test 3)

E THE INFLUENCE OF THE ADRENALS

Neuropharmacological agents are likely to interfere with the adrenal steroid hormone production In this section (exp E 1-3) the extent to which changes of the adrenal function could influence copulatory behavior in the submaximal TP treated castrated male rat was studied

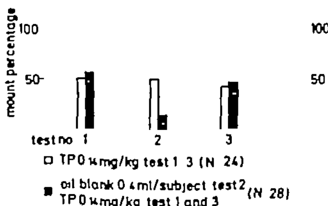


Fig 15 The effect of discontinued TP treatment on mount percentage in castrated male rats maintained on submaximal response level with respect to this paramet r by means of weekly TP injections (0 14 mg/kg) Copulatory tests were performed 3 days after the treatment Test 1 - 3 consecutive weekly tests

TABLE VII The effect of adrenalectomy on copulatory behavior in castrated male rats maintained on a submodimal response level with respect to mount percentage by means of weekly testosterone propionate treatment (0 14 mg/kg) Adrenalectomy was performed one day after the pre-experimental test followed one week later by the experimental test. Md median

Copulatory parameter	Treatment: Anesthesia Pre-exp	Exp	N	Adrenalectomy Pre-exp	Exp	N
Mount						
Percentage	53	53	36	40	37	35
Latency[Md]	20	30	15	20	20	8
No/min[Md]	2 7	2 3	15	2 3	2 7	8
Intromission						
Percentage	50	44	36	31	31	35
Latency[Md]	65	75	10	180	60	7
Ratio[Md]	74	62	10	43	40	7
Ejaculation						
Percentage	0	0	36	0	0	35

Experiment E 1 The Copulatory Response One Week After Adrenalectomy

Procedure

71 subjects in pre-experimental state III were used They had been maintained on a TP dose of 0 14 mg/kg/week since 4 months One day after the usual weekly test 35 subjects were adrenalectomized under ether anesthesia and 36 subjects were subjected just to the ether anesthesia One week after these treatments the animals were tested in the regular testing schedule after TP 0 14 mg/kg The adrenalectomized subjects were provided with 0 9% saline and tap water in their home cages

Results

The results are given in table VII One week after adrenalectomy the subjects showed a reduction in the general vigour: They moved slowly had a tangled fur and had lost about 5% of their body weight Despite the decrease in general vigour no change was seen in the copulatory parameters at that time

Experiment E 2 The Effect of Long Acting ACTH and Dexamethasone

Procedure

108 subjects in pre-experimental state III were used They had been maintained on a TP dose of 0 14 mg/kg/week for 3 months Three treatment categories were used (N = 36 for each category)

1 ACTH 20 IU/kg (third international standard slow release zinc chloride preparation)
2 free dexamethasone 2 0 mg/kg (oil suspension)
3 saline 0 4 ml/animal

The treatments were given 3 days before the copulatory tests

Results

The results are given in table VIII The saline treated subjects remained on their pre-experimental response level while significant changes occurred in both ACTH and dexamethasone treated subjects After ACTH treatment there was a significant increase in mount percentage intromission percentage and the number of mounts/min The latencies to the first mount and first intromission were significantly reduced. In contrast subjects treated with dexamethasone did not display any change in mount percentage or intromission percentage but there was an in crease in the number of mounts/min and an almost significant reduction in mount latency (p = 0 05) At the day of testing the dexamethasone treated subjects had lost about 10% of their pre-injection body weight They were restless but had in fact a decreased motor activity score compared to controls when tested for 10 min in the Animex[R] activity meter (saline = 819 ± 34 dexamethasone = 687 ± 33 mean ± s e.m. N = 9 p < 0 02)

TABLE VIII The effect of "long-acting" ACTH and free dexamethasone on copulatory behavior in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment (0 14 mg/kg) The treatments were given 3 days before the experimental test. Md = median. a $p < 0\ 05$ b = p 0 01

Copulatory parameter	Treatment: Saline 0 4 ml Pre-exp	Exp	N	Dexamethasone 2 0 mg/kg Pre-exp	Exp	N	ACTH 20 IU/kg Pre-exp	Exp	N
Mount									
Percentage	47	56	36	58	56	36	42	64[a]	36
Latency[Md]	20	35	13	25	13	18	40	20[b]	14
No/min[Md]	3 3	2 3	13	2 3	3 7[b]	17	2 5	2 9[a]	14
Intromission									
Percentage	39	42	36	44	53	36	31	58[a]	36
Latency[Md]	50	60	11	60	50	11	140	30[b]	9
Ratio[Md]	57	67	11	59	59	11	47	71	9
Ejaculation									
Percentage	0	0	36	0	3	36	0	3	36

Experiment E 3 The Effect of Short Acting ACTH in Non Adrenalectomized and Adrenalectomized Subjects

Procedure

30 non-adrenalectomized and 30 adrenalectomized subjects in preexperimental state III were used They had been maintained on a TP dose of 0 15 mg/kg for 3 and 10 months respectively Adrenalectomy was performed one month prior to this experiment. The adrenalectomized subjects were given free dexamethasone 0 2 mg/subject/week and were provided with 0 9% saline and tap water in their home cages

One half of the subjects in each category was given "short-acting" (amorphous) ACTH 10 + 5 + 5 IU/kg (third international standard) at 6 4 and 2 hours before the test respectively while the other half of the subjects was given saline at the same time intervals Three days after this test the subjects were retested.

TABLE IX The effect of "short acting" (amorphous) ACTH on copulatory behavior in non-adrenalectomized and adrenalectomized castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment (0 15 mg/kg) ACTH (10 + 5 + 5 IU/kg) or saline was given at 6 4 and 2 hours resp. before the copulatory test at Day 0 and the subjects were retested 3 days later Md median a p 0 05

Copulatory parameter	Non-adrenalectomized						Adrenalectomized					
	Day 0			Day 3			Day 0			Day 3		
	Saline	ACTH	N	Saline	ACTH	N	Saline	ACTH	N	Salin	ACTH	N
Mount												
Percentage	57	60	30	50	70	30	50	50	30	43	47	30
Latency[Md]	13	13	14	30	10	15	20	25	13	23	10	9
No/min[Md]	3 3	4 0[a]	13	3 0	3 3	15	2 3	2 0	13	2 0	2 3	9
Intromission												
Percentage	47	47	30	27	43	30	37	37	30	27	30	30
Latency[Md]	35	30	11	43	35	8	50	48	6	28	23	6
Ratio[Md]	54	53	11	57	47	8	67	69	6	53	49	6
Ejaculation												
Percentage	0	3	30	0	0	30	0	0	30	0	0	30

Two weeks later the experiment was repeated but this time the treatments were reversed; i e subjects which had received ACTH in the first test were now given saline and vice versa In the statistical analysis of this experiment the test values after saline treatment were compared to the ones after ACTH treatment.

Results

The results are given in table IX In non-adrenalectomized subjects the number of mounts/min. was significantly increased within 6 hours from the administration of ACTH while the other parameters were unchanged Three days later however mount percentage was significantly increased and mount latency significantly decreased while no other parameter was significantly changed. In adrenalectomized subjects none of these behavioral changes was observed.

F STUDIES INCLUDING OTHER BEHAVIOR PATTERNS THAN COPULATORY

This section (exp F 1) is devoted to the normal behavior displayed during copulatory tests by castrated subjects kept on a submaximal TP treatment

Experiment F 1 A Simple Way to Score the Orientation of Activity during Copulatory Tests

Procedure

179 subjects in pre-experimental state III were used They were maintained on a TP treatment of 0 10 mg/kg/week The records were taken parallel to the records of copulatory behavior The scores for the following mutually exclusive orientations of activity were recorded:

1 Activity oriented towards the female
   This includes anogenital and non-genital sniffing and interaction pursuit climbing and mounting with or without intro-

mission

2 Activity oriented towards the environment
This includes exploration of the cage rearing and other kinds of locomotor activity

3 Activity oriented towards the subject itself
This includes genital and non-genital grooming

4 Immobility
Standing still or lying down.

Scoring technique

The different orientations of activity were scored each min during the first 3 min. after the introduction of the female. Each subject was given a score of 1 for each of the 3 min. in which a certain orientation of activity was displayed The criterion for getting a score was that a certain behavior was displayed for at least 0 05 min consecutively during that min. (Exception: if a subject mounted the female it was given the score of 1 for activity oriented towards the female for that min. even if pursuit plus mount took less time than 0 05 min) During the 3 min period a subject could thus score a minimum of 0 and a maximum of 3 for each of the different orientations of activity recorded.

Subjects which performed at least one mount ("mounters) and subjects which did not mount ("non-mounters) were placed in separate categories in the analysis of the behavioral data (Subjects which ejaculated before the end of the 3 min. period were excluded.)

Results

The percentage of subjects which obtained a certain score for orientation of activity is given in fig 16 The score profiles were different for the two categories of subjects At the beginning of each test all subjects approached the female irrespective of subsequent mounting or non-mounting If they did not mount the female a major part of the first minute was devoted to anogenital sniffing of and other social interaction with her Among "mounters the most frequent score for orientation towards the female was 3

while 1 was the most frequent score among "non-mounters" Since all subjects displayed orientation towards the female during the first minute after her introduction this means that most "mounters" also paid attention to her during the next 2 min while most "non-mounters" did not display this behavior during any of the next 2 min. The average score for environment-oriented activity was greater among "non-mounters" although the most frequent score was 2 for both categories of subjects For activity oriented towards the subject itself "mounters" mainly scored 3 while non-mounters mainly scored 1 or 2 The criterion for getting a score for immobility was fulfilled by only one out of the 179 subjects This subject happened to be a non-mounter"

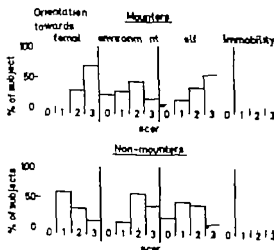


Fig 16 Orientation of tivity during copul tory te ts in castr t d mal rats maintained on a submaximal respons level with respect to mount percentage by means f weekly TP treatment (0 10 mg/kg) Comp rison is mad between mount rs and non-mount rs (N = 73 sp N = 106) A subject was given the core f 1 for ch of th 3 min in whi h a certain behavior was displayed for t l ast 0 05 min F r furth d tails: see text

DISCUSSION

The main aim of the present investigation was to find a schedule of TP treatment that induced and could maintain a submaximal response with respect to the percentage of subjects that displayed pre-ejaculation copulatory behavior The different steps of the investigation will be discussed separately

The Testing Procedure

A time schedule with an upper limit of 3 min before the fist mount and 3 min. of copulation allowed after the first mount was used in the copulatory tests This testing procedure seems to be appropriate for TP treated castrates because:

1 Within the first 2 min all but a very small number of the mounting subjects had performed their first mount (fig 10) i e mount latencies were shorter than 3 min

2 Counted from the first mount 3 min. was also a suitable time for the number of mounts/min. to be determined since: A the time was sufficient for several mounts to be performed (fig 12) and B few or no subjects ejaculated within this time (fig 14) Furthermore this short time of testing should be appropriate for pharmacological studies since:

3 The behavioral effects of even short acting pharmacological agents will change little during the course of a test

A practical consequence of this testing procedure is that:

4 Several subjects can be tested within a restricted time period (30-40/hour)

However one might assume that 3 min. of heterosexual contact/ week is such a slight reward that even an intact subject would stop copulating within a few weeks under this regimen No support for such an assumption was obtained from the present experiments Intact subjects copulated vigorously for at least 7 weeks under

this regimen (exp B2) and in fact continued to do so for more than a year (unpublished observations)

The Effect of Castration

At 5 months after castration motor activity as measured by the Animex^R activity meter was within a normal range In contrast within 7 weeks from castration copulatory activity had gradually decreased: there was a reduction in mount and intromission percentages prolonged mount and intromission latencies as well as a decreased number of mounts/min The intromission ratio however was not significantly changed indicating an adequate ability of penile erection and vaginal intromission The decreased copulatory scores after castration were not accompanied by a lack of exploring of the female at the beginning of each copulatory test.

The Effect of Single TP Doses

Regardless of the TP dose given to non-mounting subjects it took several hours (30-54 hours) until mounting behavior was displayed (exp C1) The duration of the TP effect (mount percentage) was dependent on the dose given Calculated from the day the TP injection was given the duration of the effect was more than 5 days but less than 10 days at a submaximal dose level while the duration was longer after a supramaximal dose (exp C2) The amount of TP needed for a significant change to occur was not the same for all copulatory parameters Exp C3 (and D7) shows that mount percentage and mount latency were significantly changed by a smaller dose of TP than was necessary to change the other parameters significantly

Weekly TP Injections

The choice of doses and time interval for repeated administration of TP was guided by experience obtained from the experiments with single TP injections From the results of these experiments it seemed likely that a TP dose of 0 20 mg/kg or less given repeatedly once weekly should have little or no cumulative effect with respect to mount percentage By giving weekly TP injections it was found possible to maintain a submaximal response level for several months without tendencies towards a systematic shift of the response with time (exp D3) Furthermore the level of response was dose-dependent (exp D2)

One may ask if subjects kept for long time periods on a submaximal response level remain sensitive to a change of the TP supply or if the conditioning to the regular testing procedure is so strong that a temporary change of the TP supply has little effect upon the response Exp D7 shows that still after several months of a submaximal TP treatment the copulatory response could be increased by raising the dose of TP and from exp D8 it is clear that a temporary withdrawal of the TP treatment reduced the response Thus still after several months from the beginning of treatment the subjects were sensitive to changes in the hormonal supply An injection of free testosterone (1 mg/kg) had no significant effect on the copulatory behavior within 5 hours from its administration Thus it takes more than 5 hours for testosterone to affect copulatory behavior in subjects kept on a submaximal response level with respect to mount percentage by means of weekly TP treatment.

The Influence of the Adrenals

No significant effect on the copulatory behavior was detected one week after adrenalectomy This is in accordance with earlier findings of Bloch and Davidson (1968) although their experimental ap-

proach was different Suppression of adrenal activity by means of a high dose of dexamethasone did not impair copulatory performance either On the contrary there was a significant increase in the number of mounts/min and an almost significant reduction of mount latency Since facilitatory effects of this kind were not obtained by adrenalectomy, these facilitatory effects might be due to direct central nervous effects of dexamethasone itself rather than secondary to adrenal suppression It can be concluded that if not stimulated more than ordinarily (by drugs or other factors) the adrenals seem not to produce a sufficient amount of steroid hormones to exert a major influence on copulatory behavior in the castrated male rat treated with small doses of TP On the other hand ACTH treatment brought about facilitatory effects in several respects Since a facilitation of copulatory behavior was not seen in ACTH treated adrenalectomized subjects it is likely that the facilitatory effects on copulatory behavior of systemically administered ACTH were due to an increased adrenal steroid synthesis and release Notably within 6 hours from the administration of ACTH the number of mounts/min was significantly increased The relationship between this effect and an endogenous release of testosterone could be questioned since free testosterone did not have this effect within a similar length of time Three days after the injection of short-acting" ACTH the number of mounts/min was not different from control levels but instead mount percentage was increased and mount latency decreased; such effects were also seen after a small increase of the dose of TP (exp D7) These ACTH experiments suggest that an intense stimulation of the CNS-pituitary-adrenal axis might influence copulatory behavior It is of course important to be aware of this when effects of drugs on copulatory behavior are considered especially if they are given several hours before the copulatory test

Other Behavior Patterns than Copulatory

Drugs that affect copulatory behavior in the male rat might do so either by a primary action on the physiological mechanisms which regulate this behavior and/or indirectly by an influence on e g well-boing motor capability or wakefulness Since little attention has been paid to this matter previously there is an apparent demand for simple methods to differentiate specific from non-specific effects of neuropharmacological agents on copulatory behavior The scoring technique introduced in exp F1 was developed for this purpose Regarding the orientation of activity displayed by the male rat during copulatory tests two points in particular should receive careful attention:

1 Immediately after the introduction of the female into the observation cage all males approached explored and interacted with her

2 Very few subjects fulfilled the criterion for immobility"

If a drug abolishes the initial exploring of the female or if many subjects fulfill the criterion for immobility the possibility of non-specific drug-actions on copulatory behavior should be considered

CONCLUDING REMARKS

The main objective of this investigation was to develop a method appropriate for pharmacological studies on the hormone-activated heterosexual pre-ejaculation copulatory (mounting) behavior in the castrated male rat The interest was focused on the percentage of subjects which displayed mounting (mount percentage) By means of weekly injections of small amounts of testosterone propionate mount percentage could be maintained on a stable submaximal level for several months the response remaining sensitive to changes in the hormonal supply throughout The present method seems to meet the requirements for its intended purpose Since castrates

are used drug effects on the gonadal hormone production are avoided; experimental evidence is presented however that the possibility of drug-interactions with the adrenal function should also receive attention

F r generous supplies of hormones I thank Organon the Netherlands through Erco and Pharmacia Sweden (testosterone propionate free testosterone estradiol benzoate progest rone free dexamethasone Cortrophin p ol) and Ferring Sweden (Acton) The work was supported by NIH grant R01-HD0410—03 and the Swedish Medical Research Council grant 14X-64 08 to Dr Bengt Meyerson and grants from the Medical Faculty University f Uppsala, to the author

REFERENCES

AHLENIUS S H ERIKSSON K LARSSON K MODIGH and P SÖDERSTEN Mating behavior in the male rat treated with p-chlorophenylala nine methyl ter alone and in combination with pargyline Psychopharmacologia (Berl) 1971 20 383-388

BEACH F A and A M HOLZ TUCKER Effe ts of different concentrations of andr gen upon exual behavior in castrated male rats J Comp Physiol Psychol 1949 42 433-453

BIGNAMI G Pharmacological influences o mating behavior in the mal r t Psychopharmacologia (Berl) 1966 10 44-58

BLOCH G J and J M DAVIDSON Effects of adrenalectomy and experience on postca tr tion sex behavior in th mal rat Physiol Behav 1968 3 461-465

BUTCHER L L S G BUTCHER and K LARSSON Effects of apomorphine ( ) amph tamine and nialamide on tetrabenazine-induced suppressi n f exual behavior in the male rat European J Pharmacol 1969 7 283-288

DEWSBURY D A and H W DAVIS Eff cts of reserpine on the copulatory behavior of mal r ts Physiol Behav 1970 5 1331-1333

DEWSBURY D A Effects of tetrabenazine on the copulatory beha vio of male rats European J Pharmacol 1972 17 221-226

DEWSBURY D A H M DAVIS and P E JANSEN Eff cts of monoami oxidase inhibitor on the copulatory behavi r of male rats Psychopharmacologia (Berl) 1972 24 209-217

GILLETT E Effe ts of chl rprome ine and D-lyse gic acid diethylamid on sex behavior of male rats Proc Soc Exptl Biol Med 1960 103 392-394

HYYPPÄ M P LEHTINEN and U K RINNE Effect f L DOPA on hypothalamic pineal and striatal monoamin s and n the exual behaviour f the rat Brain Research 1971 30 265-272

LEAVITT F I Drug-induced modifications in sexual behavior and open field locomotion of male rats Physiol Behav 1969 4 677-683

SALIS P J and D A DEWSBURY p-Chlorophenylalanine facilitates copulatory behaviour in male rats Nature (Lond) 1971 232 400-401

SHAPIRO H A Effect of testosterone propionate upon mating Nature (Lond) 1937 139 588-589

SIEGEL S Nonparametric statistics for the behavioral sciences (McGraw-Hill New York 1956)

SOULAIRAC M L Étude expérimentale des régulations hormono-nerveuses du comportement sexuel du Rat mâle Ann d Endocr 1963 24 Suppl to N 3 1-98

STONE C P Copulatory activity in adult male rats following castration and injections of testosterone propionate Endocrinol 1939 24 165-174

SVENSSON T H and O THIEME An investigation of a new instrument to measure motor activity of small animals Psychopharmacologia (Berl) 1969 14 157-163

TAGLIAMONTE A P TAGLIAMONTE and G L GESSA Reversal of pargyline-induced inhibition of sexual behaviour in male rats by p-chlorophenylalanine Nature (Lond) 1971 230 244-245

WHALEN R E and W G LUTTGE p-Chlorophenylalanine methyl ester: an aphrodisiac? Science 1970 169 1000-1001

ZIMBARDO P G and H BARRY Effects of caffeine and chlorpromazine on sexual behavior of male rats Science 1958 127 84-85

From the Department of Medical Pharmacology
University of Uppsala Uppsala Sweden

MONOAMINE PRECURSORS AND COPULATORY BEHAVIOR IN THE MALE RAT

By

CARL OLOF MALMNÄS

ABSTRACT

Pargyline 40 mg/kg and nialamide 250 mg/kg suppre sed testo te one-activated heterosexual copulatory behavior in castrated male rats DL 5 HTP 1 0 and 2 5 mg/kg inhibited opulatory behavior when combined with a subeffective dose of pargyline (20 mg/kg) wheres L DOPA 1 0 and 10 mg/kg did not DL 5-HTP 2 5 mg/kg inhibited copulatory behavior also when given after a combined pretreatment with pargyline (20 mg/kg) and the extracerebral d carboxyl se inhibitor MK486 50 mg/kg In contrast L-DOPA 2 5 mg/kg facilitated several components of copulatory behavio when given after the same pargyline and MK486 pretreatment while DL-DOPS 3 0 10 or 30 mg/kg did not facilitat the behavior It is sugge ted that t st sterone-activated heteros xual copulatory behavi r in th c trated mal rat is inhibited by functionally increased entral nervous ser tonin content and facilitated by increased dopamin content

INTRODUCTION

Heterosexual copulatory behavior in the male rat is dependent on gonadal hormones After castration there is a gradual decline of copulatory performance which can be restored by testosterone propionate (TP) (Shapiro 1937; Stone 1939; Beach and Holz-Tucker 1949; Malmnäs 1973a)

Recent research has demonstrated effects on certain components of copulatory behavior in intact male rats of neuropharmacological agents affecting synthesis (Tagliamonte et al 1971; Salis

and Dewsbury 1971; Ahlenius et al 1971) storage (Soulairac 1963; Butcher et al 1969; Dewsbury and Davis 1970; Dewsbury 1972) release (Soulairac 1963; Butcher et al 1969) and breakdown (Tagliamonte et al 1971; Dewsbury et al 1972) of monoamines These effects might either be due to an action of the drugs on the nervous mechanisms primarily involved in the control of this behavior and/or to other factors such as influence on e g locomotor activity or interference with the endogenous steroid hormone production.

TP treated castrated male rats were used in the present investigation The subjects were given monoamine oxidase inhibitors These were given either alone or in combination with monoamine precursor amino acids with or without an extracerebral decarboxylase inhibitor The aim was to study the effects on copulatory behavior of general and selective increases of the monoamine levels and to evaluate the specificity of the effects obtained

MATERIAL AND METHODS

A procedure has been developed to maintain castrated male rats on a submaximal response level with respect to the percentage of subjects that displayed copul tory behavior For detailed description see Malmnäs (1973a)

Experimental subjects

Exp rimental animals wer about 360 male Wistar rats (350-450 g when used in experiments purchased as Specific Pathogen Free Møllegaard Ejby Denmark) castrated as adults and with proven sexual capacity (at least one ejaculation) before castration Spayed Sprague-Dawley female rats were used as stimulus objects They were brought into behavioral estrus by 25 µg estradiol benzoate followed 48 hrs later by 1 mg progesterone s c which was given 4 7 h s before the copulatory t sts The animals were housed under forced ventilation at 21 ± 1°C and a reversed day-night light cycle (light from 10 pm to 11 am) Commercial rat pellets (Anticimex 210 Sollentuna Sweden) and tap water were supplied <u>ad lib</u>

Injected materials

Th hormones used were testosterone propionate (TP) estradiol benzoate and progesterone (Organon). The neuropharmacological compounds used were pargyline HCl (Eutonyl[R] Abbott Lab) nialamide HCl (Niamid[R] Pfizer) L-α-(3 4-dihydroxybenzyl)-α-hydrazino propionic acid (MK486 Merck Sharp & Dohme) DL-5-hydroxytryptophane (DL-5-HTP Nutritional Biochemicals Corp) L-3 4-dihydroxyphenylalanine methylester HCl (L DOPA, Hässle) and DL-threo-3 4 dihydroxyphenylserine (DL-DOPS Hässle) The hormones were dissolved in olive oil and the neuropharmacol gi al agents in saline MK486 and DL-DOPS were dissolved in hot saline to which a few drops of 2 N HCl had been added and the pH was adjusted to 3 and 5 resp by addition of 2 N NaOH All injections of hormones were given subcutaneously while the neuropharmacological agents and blank solutions were given intraperitoneally ll in a volume of 1 ml/kg Doses mentioned in the text and tables always ref r to th forms of the compounds stated above

Testing procedure

Copulatory tests were performed during the dark peri d of the light cycle (between 2 pm and 5 pm) under dimlight conditions The mal was transferred from the home cage to an observation cage (40 x 60 x 40 cm) nd stimulus female was brought in 5 min l t r The time allowed from the introduction of the female t th fi st mount was restricted to 3 min If the male mounted the femal within 3 min the test ontinued for another 3 min counted from th first mount r until ejaculation occurred whichever came fi st

Copulatory parameters recorded

Copul t ry behavi in th male rat consi ts of a serie of mounts with or without penile introm sion int the femal vagina terminated by j culation The following measu es were taken:

1 Mount percentage
Th percentage of subjects that displayed at least one mount with or without intromission; only mounts with evident pelvic thrusts were recorded

2 Mount latency
The time (expressed as hundredths of a min) from th introduction of the femal to the first mount with or without intromi sion

3 Number of mounts per min
Total number of mounts with or without intromission during the te t divided by 3 Not : subjects which ejaculated within 3 min from the first mount w r excluded from thi calculation

4 Intromission per entage
Th percentage of subject that displayed intromission within 3 min from th introduction of the female

5 Intromission latency
The time (expressed as hundredths of a min) from the introduction of the female to the first mount with intromission.
6 Intromission ratio
The percentage of mounts with intromission of the total number of mounts with or without intromission
7 Ejaculation percentage
The percentage of subjects that displayed ejaculation within 3 min from the first mount
8 Ejaculation latency
The time from the first intromission to ejaculation

Note: Parameters 2 3 5 6 and 8 were recorded for each individual subject that displayed the behavior in question while parameters 1 4 and 7 are percentage numbers based on several subjects

Experimental design of copulatory tests

Tests were carried out regularly once weekly Intact males display mounting in every test under this regimen (Malmnäs 1973a) however after castration there is a decline of the percentage of subjects which display mounting (Malmnäs 1973a) When in two successive post castration tests the mount percentage of the batch was less than 50% the animals were given TP once weekly The TP dose was adjusted to induce a stable submaximal response with respect to mount percentage (0 10 - 0 20 mg/kg 3 - 4 days before the copulatory test) In the neuropharmacological experiments the subjects were tested in groups of 9 - 12 members A group could appear in more than one treatment category but the same treatment was only given once to the same group At least one drug free test was conducted between two neuropharmacological treatments Blank treated subjects were always tested parallel to drug treated subjects as a control of the stability of the hormone-induced response When calculating statistical significance of a treatment-induced change of the behavior in the experimental test comparison was made with the last no-drug test - the "pre-experimental test - i e each subject served as its own control

Methods to evaluate the specificity of drug induced changes of copulatory behavior

A Motor activity measurements The expression of copulatory behavior in the male rat is dependent upon intact motor capabilities Motor activity measurements were made in order to find possible correlations between the effect of a drug treatment on copulatory behavior and motor activity One male at a time was run for 10 min in an Animex[R] activity meter (Svensson and Thieme 1969) (movements of an object across a tuned oscillator coil system results in a change of the tuning which is recorded as a

count) The kind of motor activity displayed during the 10 min period is mainly of the exploratory type Motor activity recordings were performed during the dark period of the day in subjects receiving the same TP and drug treatment as subjects in copulatory tests

8 Scoring of the orientation of activity during copulatory tests
The scores for the following activities were recorded parallel to the records of copulatory behavior:

1 Activity oriented towards the female
   This includes anogenital and non-genital sniffing and interaction pursuit climbing and mounting
2 Activity oriented towards the environment
   This includes exploration of the cage rearing and other kinds of locomotor activity
3 Activity oriented towards the subject itself
   This includes genital and non-genital grooming
4 Immobility
   Standing still or lying down

The occurrence of any behavior which could be assigned to one of these c tegories was scored during each of the first 3 min from the introduction of the female The criterion for getting a scor was that a certain behavior was displayed for t least 0 05 min consecutively during that min. (Exception: if a subject mounted the female it was given the score of 1 for female-oriented activity for that min. even if pursuit + mount took less time than 0 05 min) More than on orientation of ctivity could be scored each min During the 3 min. period a subject could thus score a minimum f 0 and a maximum of 3 for each of the different orientations of activity In the analysis f the behavi ral data the s bje ts were divided into two categories: 1 mounters = subjects whi h performed at least one mount both in the pre-experimental and experimental test and 2 non-mounters - ubjects which neither mounted in th pre-experimental nor in the experimental t st Subje ts which eja ulated bef re the end of the 3 min. period wer excluded from this calculation

tatistical analysis

For the analysi of the ignificance of a tr atment-induced change in the experimental t st comp red to the pre-experimental test non-parametric statistical tests we e used (Siegel 1956) namely the sign te t (mount per entage intromission percentage ejaculation per entage and orientation of activity) and the Wilcoxon matched-pairs signed-ranks test (mount late cy mounts/min intromi sion latency and intromission rati) Data which appe r in tables on mount and introml sion latencies mounts/min and intromission ratio re based on subjects which mounted and had intromisions respectively - both in the pre-exp rimental and experiment l test The Mann-Whitney U test was used for comparison between

5 Intromission latency
The time (expressed as hundredths of a min.) from the introduction of the female to the first mount with intromission.
6 Intromission ratio
The percentage of mounts with intromission of the total number of mounts with or without intromission
7 Ejaculation percentage
The percentage of subjects that displayed ejaculation within 3 min from the first mount
8 Ejaculation latency
The time from the first intromission to ejaculation

Note: Parameters 2 3 5 6 and 8 were recorded for each individual subject that displayed the behavior in question while parameters 1 4 and 7 are percentage numbers based on several subjects

Experimental design of copulatory tests

Tests were carried out regularly once weekly Intact males display mounting in every test under this regimen (Malmnäs 1973a) however after castration there is a decline of the percentage of subjects which display mounting (Malmnäs 1973a) When in two successive post-castration tests the mount percentage of the batch was less than 50% the animals were given TP once weekly The TP dose was adjusted to induce a stable submaximal response with respect to mount percentage (0 10 - 0 20 mg/kg 3 - 4 days before the copulatory test) In the neuropharmacological experiments the subjects were tested in groups of 9 - 12 members A group could appear in more than one treatment category but the same treatment was only given once to the same group At least one drug fr e test was conducted between two neuropharmacological treatments Blank treated subjects were always tested parallel to drug treated subjects as a control of the stability of the hormone-induced response When calculating statistical significance of a treatment-induced change of the behavior in the "experimental test" comparison was made with the last no-drug test - the pre-experimental test" - i e each subject served as its own control

Methods to evaluate the specificity of drug induced changes of copulatory behavior

A Motor activity measurements The expression of copulatory behavior in the male rat is dependent upon intact motor capabilities Motor activity measurements were made in order to find possible correlations between the effect of a drug treatment on copulatory behavior and motor activity One male at a time was run for 10 min in an Animex[R] activity meter (Svensson and Thieme 1969) (movements of an object across a tuned oscillator coil system results in a change of the tuning which is recorded as a

TABLE I. The effect of monoamine oxidase inhibitors on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given 6 hours before the copulatory tests Md = median. p 0 001

Copulatory parameter	Treatment mg/kg Saline 0 4 ml			Pargyline 20			Pargyline 40			Nialamide 125			Nialamide 250		
	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount															
Percentage	82	88	17	78	83	18	78	6	18	78	89	18	83	0	18
Latency Md	20	15	13	15	20	13	15	40	1	20	35	13	15		(15)
No/min Md	3 0	3 6	13	3 3	2 3	13	2 0	0 3	1	3 0	2 3	13	3 3		(15)
Intromission															
Percentage	71	59	17	72	78	18	61	0	18	61	56	18	61	0	18
Latency Md	38	35	4	28	23	10	45		(11)	70	40	7	50		(11)
Ratio Md	75	50	6	73	50	10	60		(11)	70	70	7	55		(11)
Ejaculation															
Percentage	0	0	17	0	0	18	0	0	18	0	0	18	0	0	18

TABLE II. The effect of monoamine precursors (after pargyline pretreatment) on copulatory behavior in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given at 1.5 hours (pargyline) and 0.5 hours (precursors) before the copulatory tests. Md = median.

a p < 0.05, b p < 0.01, c p < 0.001

Treatment, mg/kg				Pargyline, 20														
Copulatory parameter	Saline 2x0.4 ml			Saline 0.4 ml			L-DOPA, 1.0			+ L-DOPA, 10			+ DL-5-HTP 1.0			DL-5-HTP 2.5		
	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount																		
Percentage	72	72	36	72	75	36	78	75	36	75	75	36	81	58[a]	36	83	47[c]	36
Latency[Md]	30	38	20	35	30	21	30	20	25	50	20[b]	26	15	28	20	20	40	15
No/min[Md]	3.3	4.0	18	3.3	3.0	21	3.7	3.9	25	3.0	3.0	26	3.0	1.8[a]	20	3.3	2.3[b]	15
Intromission																		
Percentage	58	53	36	56	64	36	72	64	36	47	67	36	72	44[b]	36	64	36[a]	36
Latency[Md]	50	35	13	30	65	13	75	30	21	70	35	14	75	53	14	40	40	11
Ratio[Md]	62	53	13	40	62	13	50	60	21	39	57[b]	14	51	59	14	50	55	11
Ejaculation																		
Percentage	0	6	36	0	0	36	0	0	36	0	0	36	0	0	36	0	0	36

TABLE III. The effect of monoamine precursors (after pargyline and MK486 pretreatment) on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given at 1.5 hours (pargyline) 1.0 hours (MK486) and 0.5 hours (precursors) before the copulatory tests Md median. a p 0.05, b p 0.01, c p 0.001

Copulatory parameter	Treatment mg/kg Saline 3 x 0.4 ml			Pargyline 20 MK486 50 + Saline 0.4 ml			+ L-DOPA 25			DL-5-HTP 25		
	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount												
Percentage	58	43	38	66	63	35	56	95	39	68	37[c]	38
Latency[Md]	20	15	19	15	15	19	15	10[b]	22	13	48[b]	14
No/min[Md]	3.7	2.7	19	3.3	2.0	19	3.0	3.0	26	3.5	2.7	13
Intromission												
Percentage	37	47	38	40	37	35	41	67[b]	39	55	32[a]	38
Latency[Md]	35	25	13	35	43	10	30	15[b]	15	30	60[a]	11
Ratio[Md]	50	44	13	34	39	10	60	75	15	42	55	11
Ejaculation												
Percentage	0	0	38	0	0	35	0	18[b]	39	3	0	38

TABLE IV The effect of DL-DOPS (after pargyline and MK486 pretreatment) on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given at 1 5 hours (pargyline), 1.0 hours (MK486) and 0 5 hours (DL-DOPS) before the copulatory tests Md median. * p 0 05

Copulatory parameter	Treatment mg/kg											
				Pargyline 20 MK486 50								
	Saline 3 x 0 4 ml			DL-DOPS 3 0			DL-DOPS 10			DL-DOPS 30		
	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount												
Percentage	55	52	42	37	41	27	47	47	36	53	57	30
Latency Md	15	15	17	20	10	7	13	10	14	13	40*	12
No/min Md	2 3	2 0	17	2 3	1 7	7	2 3	2 0	13	2 3	1 7*	12
Intromission												
Percentage	38	36	42	22	30	27	33	44	36	37	37	30
Latency Md	85	60	9	50	110	4	75	28	8	50	60	6
Ratio Md	40	56	9	62	40	4	64	58	8	51	45	6
Ejaculation												
Percentage	2	0	42	0	0	27	0	3	36	0	0	30

cant effect on the copulatory parameters recorded (table II)

L-DOPA 1 0 mg/kg did not significantly change any of the copulatory parameters However after L-DOPA, 10 mg/kg there was a decreased mount latency and an increased intromission ratio In contrast DL-5-HTP 1 0 or 2 5 mg/kg significantly decreased mount percentage intromission percentage and the number of mounts/min. No other parameter was significantly changed.

The higher dose of L-DOPA, 10 mg/kg was the only treatment that evidently affected the overt behavior With this treatment the subjects had a reduced locomotor activity (see below) and their furs showed piloerection and were slightly wet

The Effect of Monoamine Precursor Amino Acids after Extracerebral Decarboxylase Inhibition

Due to its poor passage through the blood-brain barrier the decarboxylase inhibitor MK486 more effectively hinders the peripheral increase in monoamine formation after precursor treatment than the central nervous utilization of the precursor (Porter et al 1962; Bartholini and Pletscher 1969; Henning and Rubenson 1971) MK486 was used to investigate the significance of increased central nervous versus peripheral monoamine levels for the copulatory behavior The experiments were designed analogously to the pargyline + precursor experiments but MK486 was given as well

Pargyline 20 mg/kg given 1 5 hours before test followed by MK486 50 mg/kg 1 0 hours before test had no significant effect when combined with saline given 0 5 hours before test (table III) When L-DOPA, 2 5 mg/kg was added to this pargyline + MK 486 pretreatment mount percentage and intromission percentage increased significantly Furthermore mount latency and intromission latency were reduced and intromission ratio was increased. Despite the fact that there was no significant increase of the number of mounts/min. 7 subjects achieved ejaculation (median ejaculation latency 2 1 min) whereas no one ejaculated in the pre-experimen-

tal test. The subjects that achieved ejaculation did not have more intromissions in the experimental test than they had in the pre-exp test Quite different effects were obtained by addition of DL-5-HTP 2 5 mg/kg instead of L-DOPA. After DL-5-HTP 2 5 mg/kg mount percentage and intromission percentage were significantly decreased and mount latency was increased No significant effects were obtained on the other parameters recorded.

There were no signs of peripheral effects of the precursors among the subjects in these experiments

The Effect of DL DOPS

DL-DOPS was given after the same combined pargyline + MK486 pretreatment as in the preceding experiment in the doses 3 0 10 or 30 mg/kg At none of these dose levels was there any facilitatory effect on any of the behavioral parameters recorded (table IV) At the highest dose tested 30 mg/kg mount latency was significantly increased and the number of mounts/min. was decreased The predominant change of the overt behavior was a dose-dependent decrease of locomotor activity (see below) At even higher doses (60 mg/kg) the decrease in motor activity was still more pronounced

Motor Activity

Pargyline 40 mg/kg increased motor activity (table V) while pargyline 20 mg/kg (1 5 hours) decreased motor activity when given either with or without MK486 although only the latter combined treatment induced a statistically significant difference L-DOPA 10 mg/kg after pargyline alone and 2 5 mg/kg after pargyline + MK486 induced a significant further decrease in motor activity An addition of DL-DOPS 30 mg/kg also caused a further decrease in motor activity while no further decrease in motor activity was

TABLE V The effect of certain drug treatments on motor activity in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. One animal at time was run for 10 min. in an Animex activity meter beginning at zero hour Statistical evaluation was performed with the Mann-Whitney U test.

MOTOR ACTIVITY

	Treatment mg/kg	At hour	Counts/10 min ± m	N
A1	Saline 0 4 ml	6	780 68	6
A2	Pargyline 40	6	1138 40	6
B1	Saline 0 4 ml	1 5 -0 5	821 ± 63	9
	Pargyline 20	1 5		
B2	Saline 0 4 ml	0 5	645 − 36	9
B3	+ DL 5-HTP 2 5	0 5	683 67	9
B4	L-DOPA 2 5	0 5	457 67	9
C1	Saline 0 4 ml	1 5 1 0 0 5	826 ± 40	8
	Pargyline 20	1 5		
	MK486 50	1 0		
C2	Saline 0 4 ml	0 5	662 ± 31	6
C3	DL-5-HTP 2 5	0 5	700 27	6
C4	L-DOPA 2 5	0 5	497 55	6
C5	DL-DOPS 10	0 5	610 + 38	7
C6	DL DOPS 30	0 5	515 ± 42	6

Probabilities associated with the scores of the different treatment groups

Groups compared	p	Groups compared	p	Groups compared	p
A1 and A2	0 01	C1 and C2	0 01	C2 and C5	NS
B1 and B2	NS	C1 and C3	0 05	C2 and C6	<0 05
B1 and B3	NS	C1 and C4	0 001	C3 and C4	0 01
B1 and B4	<0 05	C1 and C5	0 01	C3 and C6	0 01
B2 and B3	NS	C1 and C6	0 001	C4 and C6	NS
B2 and B4	0 05	C2 and C3	NS	C5 and C6	NS
B3 and B4	NS	C2 and C4	0 05		

TABLE VI. Comparison of the effect of certain drug treatments on mount percentage and motor activity + increased, decreased. NS = not significant (p 0 05) one symbol p 0 05 two symbols = p 0 01 three symbols = p 0 001 Statistics mount percentage = difference between pre-experimental and experimental test, motor activity = difference between drug and saline treated subjects.

	DRUG TREATMENT	MOUNT PERCENTAGE	MOTOR ACTIVITY
A1	Pargyline 40 mg/kg		+ +
	Pargyline 20 mg/kg		
B1	+ Saline 0 4 ml	NS	NS
B2	+ DL 5-HTP 2 5 mg/kg	- -	NS
B3	+ L DOPA 10 mg/kg	NS	
	Pargyline 20 mg/kg		
	MK486 50 mg/kg		
C1	Saline 0 4 ml	NS	-
C2	DL 5-HTP 2 5 mg/kg		NS
C3	L-DOPA 2 5 mg/kg	+	-
C4	DL-DOPS 30 mg/kg	NS	

detected after addition of DL-5-HTP 2 5 mg/kg to either pretreatment. For comparison between the effects of certain drug treatments on mount percentage and motor activity table VI was constructed It is apparent that a change in one behavior is not associated with a corresponding change in the other behavior

Scoring of the Orientation of Activity during Copulatory Tests

Each subject was given the score of 1 for each of the 3 min in which a certain orientation of activity was displayed for at least 5 hundredths of a min consecutively The following classes of behavior were distinguished: Activity oriented 1 towards the female 2 towards the environment 3 towards the subject itself and 4 immobility This procedure was developed during the course of this investigation and was only applied to the precursor experiments with the combined pargyline + MK486 pretreatment The results are given in tables VII (pre-experimental scores) and VIII Irrespective of subsequent mounting or non-mounting female-oriented activity (enough to be scored) was displayed by all subjects during the first min of the test Thus the change of scores for female-oriented activity only reflect the change obtained during the last two min During these two min the scores for female oriented activity were decreased among DL-5-HTP treated non-mounters (but not in mounters) Environment-oriented activity was increased among DL-DOPS 30 mg/kg treated mounters but not significantly changed among other treatment categories The only significant change of scores for self-oriented activity was a decrease among DL-5-HTP treated mounters The occurrence of immobility was a conspicuous effect among L-DOPA treated subjects Seventeen out of 39 L-DOPA treated subjects got a score for immobility during at least one min. in the exp test whereas none of these subjects got any score in the pre-exp test ($p < 0\ 001$) This immobility was characterized by periods of 10 - 25 hundredths of a min. during which the subjects stopped ongoing activity just standing still It is of particular interest that a facilitation of the copulatory behavior could occur in spite of this immobility In other experiments it was found that this kind of immobility was more pronounced after L-DOPA 5 0 mg/kg and less pronounced after L-DOPA 1 0 mg/kg than it was after L-DOPA 2 5 mg/kg as used in the present experiments

TABLE VII. Orientation of activity during copulatory tests in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The table shows the percentage distri bution of pre-experimental scores Each subject was given the score of 1 for each of the 3 min. in which certain behavior was displayed for at least 0 05 min. consecutively

Percentage di tribution f pre-experimental scores

	Orientation toward															
	femal				environment				self				Immobility			
Score	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Mounters (N 116)	0	3	19	78	36	35	22	6	3	21	22	55	100	0	0	0
Non-mounters (N 86)	0	59	33	8	1	7	55	37	24	49	22	5	100	0	0	0

TABLE VIII. The effect of monoamine precursors (after pargyline and MK486 pretreatment) on orientation of activity during copulatory tests. The table shows the number of subjects which in the experimental test received [illegible] score different from the one in the p[illegible] test. Scoring technique see table VII and Methods

Treatment (mg/kg)	Orientation toward femal			environment			self			Immobility		
	Direction of changed score in the exp test and the associated probability											
			p		+	p		+	p		+	p
Saline 0 4 ml												
mounters (N 36)	7	4	NS	10	12	NS	9	10	NS	0	0	NS
non-mounter (N 25)	7	5	NS	7	7	NS	7	7	NS	0	0	NS
Pargyline 20 + MK486 50												
Saline 0 4 ml												
mounters (N 19)	3	2	NS	4	7	NS	6	6	NS	0	0	NS
non-mounter (N 9)	0	3	NS	4	2	NS	4	0	NS	0	0	NS
DL-5-HTP 2 5												
mounter (N 13)	3	3	NS	3	3	NS	6	0	0 05	0	0	NS
non-mounters (N 12)	8	1	0 05	5	3	NS	5	5	NS	0	0	NS
+ L-DOPA 2 5												
mounters (N 16)	2	3	NS	5	5	NS	2	5	NS	0	7	0 01
non-mounters (N 2)	1	0	NS	1	0	NS	0	2	NS	0	0	NS
DL-DOPS 3 0												
mounter (N 7)	4	0	NS	1	4	NS	4	0	NS	0	0	NS
non-mounters (N 13)	2	2	NS	3	4	NS	3	3	NS	0	0	NS
+ DL-DOPS 10												
mounters (N = 13)	2	3	NS	4	4	NS	3	4	NS	0	1	NS
non-mounter (N 16)	5	2	NS	2	5	NS	3	4	NS	0	1	NS
+ DL-DOPS 30												
mounters (N 12)	5	1	NS	1	8	0 05	6	3	NS	0	0	NS
non-mounters (N 9)	2	3	NS	2	2	NS	0	5	NS	0	0	NS

DISCUSSION

This investigation demonstrates that the two monoamine oxidase inhibitors pargyline and nialamide inhibit testosterone-activated heterosexual copulatory behavior in the castrated male rat. The specificity of this inhibitory effect is however difficult to evaluate as it was associated with evident effects on gross behavior Similar inhibitory effects of monoamine oxidase inhibitors on copulatory behavior in the intact male rat have recently also been reported by Tagliamonte et al (1971) and Dewsbury et al (1972)

The mount and intromission percentages as well as the number of mounts/min. were significantly decreased by DL-5-HTP given after a dose of pargyline which alone did not influence the copulatory behavior In contrast L-DOPA given in an analogous experiment did not inhibit the copulatory response although the motor activity was decreased more by that treatment than by DL-5-HTP This suggests that the antagonistic effect on copulatory behavior by a high dose of pargyline and nialamide was predominantly due to an increased serotoninergic tone (In the following the term serotoninergic tone will be used but the possibility exists that other metabolites of 5-HTP than serotonin might contribute to this inhibitory effect) That the inhibitory effect of pargyline is due to an increased serotoninergic tone is further supported by the fact that pretreatment with the serotonin-synthesis inhibitor p-chlorophenylalanine prevents pargyline from inhibiting copulatory behavior (Tagliamonte et al 1971; Malmnäs and Meyerson 1971) If DL-5-HTP is added to the combined p-chlorophenylalanine + pargyline treatment the inhibitory effect of pargyline is re-established (Malmnäs and Meyerson 1971) The experiments with the extracerebral decarboxylase inhibitor MK486 given in addition to the pargyline + precursor treatments demonstrate that central nervous rather than peripheral serotonin exerts the inhibitory effect. In these experiments the difference between the effects of DL-5-HTP

and L-DOPA on copulatory behavior became still more pronounced In fact the effects of the two precursors were opposite to each other with respect to mount percentage mount latency intromission percentage and intromission latency

With the testing procedure used in this investigation only a very small number of subjects normally achieve ejaculation within the time of a test session It is therefore only possible to detect drug induced decreases of the ejaculation latencies Ejaculation was achieved by a significant number of subjects after treatment with pargyline + MK486 + L-DOPA which indicates a facilitation of central nervous mechanisms involved in the triggering of ejaculation This finding also suggests that the reduction of ejaculation latency by D-amphetamine reported by Butcher et al (1969) may at least in part be due to an increased central nervous catecholaminergic tone

Although L-DOPA facilitates several components of the copulatory behavior after combined pargyline + MK486 pretreatment efforts in this laboratory to obtain similar effects of L-DOPA after extracerebral decarboxylase inhibition but without pargyline pretreatment have as yet failed (unpublished) This failure is in accordance with recently reported results on the effect of L-DOPA after extracerebral decarboxylase inhibition on copulatory behavior in intact male rats (Hyyppä et al 1971) Several reasons for this discrepancy can be proposed such as a displacement of serotonin by metabolites of the higher doses of L-DOPA necessary to obtain a similar increase in the catecholaminergic tone or a different distribution within the central nervous system of the newly formed catecholamines after pargyline pretreatment than without this pretreatment

Regarding the specificity of the opposite effects of L-DOPA and DL-5-HTP on copulatory behavior as demonstrated in this investigation motor activity measurements and scoring of the activity during copulatory tests give no reason to believe that these differences are merely secondary to changes of the general activity of the subjects Since L-DOPA facilitated copulatory behavior

while causing significant reduction in motor activity and even periods of immobility it is obvious that there is a great degree of specificity with respect to the facilitatory effects on copulatory behavior No such motor activity changes were detected after DL-5-HTP treatment in the doses used. Concerning the orientation of activity during copulatory tests it is of particular interest that the scores for female-oriented activity were significantly decreased among DL-5-HTP treated non-mounters This finding indicates that other components of the male rat s interaction with the female than copulatory are also inhibited by DL-5-HTP

Since small amounts of the precursors DL-5-HTP and L-DOPA were used in the present investigation and since opposite effects were obtained on the copulatory behavior displacement of catecholamines by serotonin or of serotonin by catecholamines should not be of any major significance It is likely that the effects obtained by DL-5-HTP are due to an increased serotoninergic tone and the effects obtained by L-DOPA due to an increase of the catecholaminergic tone

In analogous experiments no facilitatory effects were seen after treatment with the noradrenaline precursor DL-DOPS Possibly no functionally effective increase of the central nervous noradrenaline content was achieved. However, like L-DOPA DL-DOPS decreased motor activity The absence of facilitatory effects after DL-DOPS and the increase in mount latency (in contrast to the decreased mount latency by L-DOPA) favours dopamine rather than noradrenaline as a neurotransmitter mediating a facilitation of testosterone-activated heterosexual copulatory behavior in the castrated male rat A facilitatory function of dopamine is also suggested from other findings previously reported from this laboratory: Copulatory behavior was inhibited by the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine but not by the dopamine β-hydroxylase inhibitor FLA-63 and it was facilitated by the dopamine receptor stimulating agent apomorphine but not by the noradrenaline receptor stimulating agent clonidine (Malmnäs and Meyerson 1972; see also Malmnäs 1973c d)

For generous supplies of hormones and drugs I thank Organon the Netherlands through Erco Sweden (testosterone propionate estradiol benzoate and progesterone) Dr H Corrodi Hässle Sweden (L-3 4-dihydroxyphenylalanine methylester HCl DL-threo-3 4-dihydroxyphenylserine) Merck Sharp & Dohme International New Jersey (MK486) Abbott Lab Illinois (pargyline HCl) and Pfizer New York (nialamide HCl) The work was supported by NIH grant R01-HD04108-03 and the Swedish Medical Research Council grant 14X-64-08 to Dr Bengt Meyerson and grants from the Medical Faculty University of Uppsala to the author

REFERENCES

AHLENIUS S H ERIKSSON K LARSSON K MODIGH and P SÖDERSTEN Mating behavior in the male rat treated with p-chlorophenylalanine methyl ester alone and in combination with p rgyline Psychopharmacologia (Berl) 1971 20 383-388

BARTHOLINI B and A PLETSCHER Effect of varion decarboxylase inhibitors on the cerebral metabolism of dihydroxyphenylalanine J Pharm. Pharmacol 1969 21 323-324

BEACH F A and A M HOLZ-TUCKER Effects of diff rent concentrations of androg n upon sexual behavior in castrated male rats J Comp Physiol Psychol 1949 42 433-453

BUTCHER L L S G BUTCHER and K LARSSON Effects of apomorphine (+) amph tamine and nialamide on tetrabenazine-induced suppr ssion of sexual behavior in the male rat European J Pharmacol 1969 7 283-288

DEWSBURY D A and H.M DAVIS Eff cts of res rpine on th copulatory b havior of mal rats Physiol Behav 1970 5 1331-1333

DEWSBURY D A Eff cts f tetrabenzaine on the copul tory be havi r f mal rat European J Pharmacol 1972 17 221-226

DEWSBURY D A H M DAVIS and P E JANSEN Eff cts of monoamine oxidase inhibitors on the copulat ry behavior f male rats Psychopharmacologia (Berl) 1972 24 209-217

HENNING M and A RUBENSON A Eff cts of 5-hydroxytryptophane on arteri l bl od pres ure body temperature and ti sue monoamines in the rat Acta pharmacol (Kbh) 1971 29 145-154

HYYPPÄ M P LEHTINEN and U K RINNE Effect f l-dopa on the hypothalamic pineal and striatal monoamines and on th sexual behavi of th rat Brain Research 1971 30 265-272

MALMNÄS C O and B J MEYERSON p-Chl rophenylalanine and copulatory behaviour in the mal rat Nature 1971 232 398-400

MALMNÄS C O and B J MEYERSON Monoamines and copul tory activation in th castrated male t Acta Pharmacol et Toxicol 1972 31 Suppl 1 23

MALMNÄS C O Testo t rone-a tivated copul t ry behavio in the castrated mal rat Acta physiol scand 1973a Suppl 395 9-46

MALMNÄS C O Copulatory behavior in the male rat after impaired monoaminergic neurotransmission Acta physiol scand. 1973c Suppl 395 69-95
MALMNÄS C O Effects of LSD-25 clonidine and apomorphine on copulatory behavior in the mal rat Acta physiol. scand 1973d. Suppl 395 96-116
PORTER C C L S WATSON D C TITUS J A TOTARO and S S BYER Inhibition of dopa-decarboxylase by the hydrazine analog of α-methyldopa Biochem. Pharmacol 1962 11 1067-1077
SALIS P J and D A DEWSBURY p-Chlorophenylalanine facilitates copulatory behaviour in male rats Nature 1971 232 400-401
SHAPIRO H A Effect of testosterone propionate upon mating Nature 1937 139 588-589
SIEGEL S Nonparametric statistics for the behavioral sciences (McGraw-Hill New York 1956)
SOULAIRAC M L Étud expérimental des régulations hormononerveuses du comportement sexuel du rat male Ann d Endocr 1963 24 Suppl to N 3 1-98
STONE C P Copulatory activity in adult male rats following castration and injections of testost rone propionate Endocrinol 1939 24 165-174
SVENSSON T H and G THIEME An investigation of a new instrument to measure motor activity of small animals Psychopharmacologia (Berl) 1969 14 157-163
TAGLIAMONTE A P TAGLIAMONTE and G L GESSA Reversal of pargyline-induced inhibition of sexual behaviour in male rat by p-chlorophenylal nine Nature 1971 230 244-245

From the Department of Medical Pharmacology
University of Upps la Uppsala Sweden

COPULATORY BEHAVIOR IN THE MALE RAT AFTER IMPAIRED MONOAMINERGIC NEUROTRANSMISSION

By

CARL OLOF MALMNÄS

ABSTRACT

The two monoamine depletors reserpine 0 5 mg/kg and tetrabenaz- ine 1 0 and 3 0 mg/kg suppre sed te t sterone- tivated h tero- sexual copul tory behavior in ca trated mal rats A decrease of th copulatory espons was also seen after the c techolamine syn- thesi inhibit r α-methyl-p-tyr ine 75 and 150 mg/kg In cont- t th serotonin synthesis inhibitor p-chlorophenylalanine 4 x 100 mg/kg facilit ted the re ponse Th dopamine β hydroxylase inhibitor FLA-63 10 mg/kg did not d creas the copul tory res- pons Th perc ntage f ubj ct which initiated copulatory be- havi r was decrea ed ft r the neuroleptics chlorpromazine 1 0 and 2 5 mg/kg and pimozide 0 25 mg/kg but not afte the nor- adrenaline cept r blocking gent ph noxyb nzamin 1 0 and 3 0 mg/kg or th ad nergic β-receptor blocking agent propranolol 1 0 and 4 0 mg/kg It is suggested that test sterone-activated heterosexual copulatory behavior in th castrated male rat is fa- ilitat d by decreas d serotonin rgi ton and inhibited by de- reased dopaminergic tone

INTRODUCTION

Recent research has demonstrated that p-chlorophenylalanine (PCPA) facilitates components of heterosexual copulatory behavior in the intact (Sheard 1969; Tagliamonte et al 1971; Salis and Dewsbury 1971; Ahlenius et al 1971) and castrated (Malmnäs and Meyerson

1971) male rat as well as male-to-male mounting in the intact (Tagliamonte et al 1969; Shillito 1970; Mitler et al 1972) and castrated (Gessa et al 1970; Malmnäs and Meyerson 1971; Bond et al 1972) male rat. These behavioral effects are most likely due to the predominant effect of PCPA namely a decrease of the biosynthesis of serotonin (Koe and Weissman 1966; Jequier et al 1967)

In contrast to the great interest devoted to the effect of PCPA, little attention has been paid to the effect on copulatory behavior of drugs with a selective influence on catecholaminergic neurotransmission

The aim of the present investigation was to study the effect on copulatory behavior of drugs which induce a general or selective decrease of the monoaminergic tone and to evaluate the specificity of the effects obtained. Castrated male rats supplied with small amounts of testosterone propionate (TP) were used in order to avoid drug interactions with the gonadal hormone production and to be able to maintain a submaximal response level in the pre-drug state

MATERIAL AND METHODS

A procedure has been developed to maintain castrated male rats on a submaximal response level with respect to the percentage of subjects which displayed copulatory behavior (Malmnäs 1973a) The method is also described in detail in Malmnäs (1973b) from whi h no change has been made (See page 48 in this supplement)

Subjects

Experimental subjects were about 450 male Wistar rats castrated as adults In one experiment bilaterally adrenalectomized castrated subjects were used which besides tap water also were supplied with 0 9 saline in their home cages and were injected once weekly with free dexamethasone 0 5 mg/animal given at the same day as TP

Injected materials

The hormones used were testosterone propionate (TP) estradiol benzoate progesterone and free dexamethasone (Organon) The neuropharmacological compounds used were reserpine (Serpasil[R] Ciba ampoules 2 5 mg/ml) tetrabenazine HCl (Nitoman[R] Hoffman-La Roche) dl-p-chlorophenylalanine methylester HCl (PCPA H69/17 Hässle) dl-α-methyl-p-tyrosine methylester HCl (αMT H44/68 Hässle) bis(4-methyl-1 homopiperazinylthiocarbonyl)-disulphide (FLA-63 H83/39 Hässle) chlorpromazine HCl (Hibernal[R] Leo ampoul s 25 mg/ml) pimozide (Janssen) phenoxybenzamin HCl (Dibenyline[R] Smith Kline & French) and dl-propranolol HCl (Inderal[R] ICI) The hormones were dissolved (or, dexamethasone suspended) in olive oil and the neuropharmacological agents dissolved or diluted in s line (except reserpine which was diluted with distilled water) Pimozide was dissolved in glacial acetic acid diluted with saline and the pH adjusted to 5 by addition of 2 N NaOH Phenoxybenzamine was dissolved in hot acid propylene glycol (1% solution) and diluted with saline immediately before injection Hormones were given subcutaneously whil the neuropharmacological agents and saline were given intraperitoneally all in a volume of 1 ml/kg (Exception: propranolol 4 mg/kg was given 1 ml/kg i p and 3 ml/kg s c) Doses menti ned in the t xt and tables always refer to the forms of the compounds stated above

Three classes of neuropharmacological agents have been used: 1 Monoamine depletors (res rpine tetrabenazine) 2 Monoamine synthesis inhibitors (PCPA αMT FLA-63) and 3 Monoamine receptor blocking agents (chlorpromazine pimozide phenoxybenzamine and propranolol) Whereas αMT inhibits the catecholamine synthesis at the tyrosin hydroxylase level (Spe t r et al 1965) FLA 63 inhibits the enzyme that converts dopamine to noradrenaline dopamine β hydroxylase (Florvall and Corrodi 1970) Chlorpromazine blocks both dopamine and noradrenaline receptors (Andén et al 1970) pimozid blocks d pamine but not noradrenaline receptors (Andén t al 1970) phenoxybenzamine blocks noradrenaline but not dopamine receptors (Andén t al 1966 1967 re p 1966a) while propranol l block adrenergic β-receptors (Black et al 1964)

RESULTS

The Effect of Reserpine

Thirty hours after reserpine 0 5 mg/kg the mount percentage and intromission percentage were significantly lower than in the pre-exp test (table IA) Furthermore mount latency was increased

TABLE I The effect of reserpine and tetrabenazine on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline treatments were given 30 hours (reserpine A) or 2 hours (tetrabenazine B) before the experimental tests Md median.

p 0.05 b p 0 01, c p 0 001.

Copul tory parameter	Tr treat mg/kg A1 Saline 0 4 ml Pre-exp	Exp	N	A2 Re erpine 0 5 Pre-exp	Exp	N	B1 Saline 0 4 ml Pre-exp	Exp	N	B2 Tetr bena ine 1 0 Pre-exp	Exp	N	B3 Tetrabena ine 3 0 Pre-exp	Exp	N
Mount															
Pe centage	50	53	32	54	33	39	53	50	32	51	30^{a}	45	44	14^{b}	36
Latency^{Md}	13	13	12	15	45	12	20	20	14	18	28	16	20	10	4
No/min^{Md}	3 3	3 0	11	2 7	1 0^{b}	11	3 0	2 5	14	2 5	2 0	16	2 8	2 3	4
Intromission															
Percentage	41	44	32	38	15^{b}	39	44	34	32	33	27	45	28	11	36
Latency^{Md}	50	15	8	15	118	6	35	40	8	40	100	8	35	15	3
Ratio^{Md}	47	38	8	63	50	6	56	47	8	65	58	8	50	56	3
Ejacul tion															
Percentage	3	3	32	3	0	39	0	0	32	0	0	45	0	0	36

and the number of mounts/min decreased Changes of the other parameters did not reach statistical significance

The subjects had an evident ptosis and a reduced motor activity However, they displayed a normal non-copulatory interaction with the female (see below)

The Effect of Tetrabenazine

A significant decrease of mount percentage was obtained by tetrabenazine 1 0 and 3 0 mg/kg given 2 hours before the copulatory tests (table IB) Other parameters were not significantly changed.

The overt behavior was little affected by tetrabenazine 1 0 mg/kg but after 3 0 mg/kg the subjects sometimes stopped ongoing activity just standing still for short periods (0 10 - 0 30 min.) and had a decreased motor activity However the subjects displayed a normal amount of anogenital sniffing and pursuit of the female (see below) Neither ptosis nor hunched backs were noticed in these subjects

The Effect of PCPA

PCPA 100 mg/kg was given either as a single dose 6 hours before the copulatory test or repeatedly for 4 consecutive days the last injection 6 hours before the copulatory test Six hours after PCPA 100 mg/kg neither mount percentage nor intromission percentage was significantly changed (table IIA) The only significant change of the copulatory parameters was a decreased mount latency In contrast when 100 mg/kg of PCPA was given for 4 consecutive days a clearcut increase in mount and intromission percentages a decreased mount latency and an almost significant (p = 0 05) increase in intromission ratio was seen 6 hours after the last injection A striking effect of the PCPA treatment was the occurrence of ejaculation; whereas no subject ejaculated in the pre-exp

TABLE II The effect of PCPA on copulatory behavior in non-adrenalectomized (A) and adrenalectomized (B) castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline treatments were given at 78 54 30 and 6 hours (or A2 at 6 hours) before the experimental tests

Md median, a p 0 05 b p 0 01, c p 0.001

Copulatory	Treatment mg/kg														
	A1			A2			A3			B1			B2		
	Saline 4 0 4 ml			PCPA 100			PCPA 4 100			Saline 4x0 4 ml			PCPA 100+3 50		
parameter	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount															
Percentage	45	45	40	47	42	38	49	90[c]	39	35	35	34	31	80[c]	35
Latency[Md]	20	25	15	15	10	11	25	15	19	33	20	6	30	10[b]	10
No/min[Md]	4 0	3 0	15	3 0	2 7	11	2 0	4 2	4	2 2	3 0	6	1 7	3 3	10
Intromission															
Percentage	40	40	40	29	32	38	38	82[c]	39	18	29	34	14	57	35
Latency[Md]	20	33	13	25	15	7	40	20	15	25	35	3	35	40	5
Ratio[Md]	62	56	13	29	57	7	61	75	15	38	31	3	71	57	5
Ejaculation															
Percentage	0	0	40	0	0	38	0	51[c]	39	0	0	34	0	0	35

test 20 out of 39 subjects ejaculated in the exp test (13 out of 15 subjects which achieved intromission in the pre-exp test) None of the controls ejaculated The median ejaculation latency after PCPA treatment was 2 05 min The absolute number of mounts with or without intromission was not significantly different from the pre-exp test but they were achieved during a shorter time and thus at a faster rate

The main effect on the overt behavior was a decreased motor activity (see below)

The Effect of PCPA in Adrenalectomized Subjects

Since ACTH given subcutaneously brought about a facilitation of certain components of the male copulatory behavior in methodologically analogous experiments (see Malmnäs 1973a) it was of interest to study the effect of PCPA in adrenalectomized subjects PCPA 100 mg/kg was given one day followed by 50 mg/kg for 3 consecutive days the last injection 6 hours before the copulatory tests As is shown in table IIB PCPA induced a clearcut increase in mount and intromission percentages as well as in the number of mounts/min and there was a significantly decreased mount latency There were no ejaculations however

Among the PCPA treated subjects the gross appearance in spite of the dexamethasone treatment was affected: the subjects had tangled furs and hunched backs

The Effect of αMT

αMT 75 mg/kg given 6 hours before the copulatory tests induced a significant decrease in mount percentage and intromission percentage an increased mount latency and a decreased number of mounts/min. (table IIIA) The other parameters were not significantly changed.

TABLE IIIA. The effect of αMT on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given at 6 hours before the experimental tests Md median.
p 0 05 b = p 0 01 p 0 001

	Treatment mg/kg					
	A1			A2		
Copulatory	Saline 0 4 ml			αMT 75		
Paramet r	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount						
Percentage	59	56	34	60	37[b]	35
Latency[Md]	18	13	14	15	80	13
No/min[Md]	3 0	2 7	14	3 0	1 7[b]	13
Intromi sion						
P rcentage	41	35	34	46	9	35
Lat ncy[Md]	15	20	9	10	40	3
Ratio[Md]	50	45	9	38	69	3
Ejaculation						
Percentag	0	0	34	0	0	35

No obvious effect on the overt behavior was seen (see below)

αMT 150 mg/kg was given 3 or 6 hours before the copulatory tests (table IIIB) This dose of αMT decreased mount percentage and in the 6 hour experiment also the intromission percentage The small number of mounting subjects in the exp test does not permit an estimate of the effects on other copulatory parameters

In the 3 hour experiment the locomotor activity was reduced but otherwise no obvious change of the overt behavior was seen In the 6 hour experiment the subjects had a tendency to stop ongoing activity standing still in a hunched back posture

TABLE IIIB. The effect of oMT on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given at 6 hours or B2 at 3 hours before the experimental tests Md median
b p 0 01, p 0 001

Copulatory parameter	Treatme t, mg/kg B1 Saline 0 4 ml			B2 oMT 150			B3 oMT 150		
	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount									
Percentage	43	45	47	55	18[b]	22	58	8[c]	24
Latency[Md]	33	40	15	15	65	4	13	120	2
No/min[Md]	3 2	2 5	15	1 9	1 7	4	4 2	1 7	2
Intromission									
Percentage	36	38	47	32	14	22	42	4[b]	24
Latency[Md]	50	60	11	20	80	3	40	200	1
Ratio[Md]	50	50	11	56	50	3	58	33	1
Ejacul tion									
Percentag	0	0	47	0	0	22	0	0	24

TABLE IV The effect of FLA-63 on copulatory behavior in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline treatments were given 4 hours before the experimental tests
Md median. p 0 05

Copulatory parameter	Treatment mg/kg Saline 0 4 ml Pre-exp	Exp	N	FLA 63 10 Pre-exp	Exp	N
Mount						
Percentage	53	53	40	55	48	33
Latency[Md]	15	10	17	18	20	14
No/min[Md]	2 7	2 3	15	2 0	3 3	13
Intromission						
Percentage	35	35	40	24	36	33
Latency[Md]	30	20	9	45	40	6
Ratio[Md]	50	55	9	52	63	6
Ejaculation						
Percentage	0	5	40	6	3	33

The Effect of FLA-63

FLA-63 10 mg/kg given 4 hours before the copulatory tests had no inhibitory effect on the copulatory behavior (table IV) On the contrary the number of mounts/min was increased

FLA-63 10 mg/kg decreased locomotor activity (see below) and the subjects had a slight but evident ptosis At a higher dose (25 mg/kg) the subjects sat immobile crouched and did not take any notice of the female

TABLE V The effect of chlorpromazine on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline injections were given 1 hour before the experimental tests Md median. b p 0 05 c p 0.001

Copulatory parameter	Treatment mg/kg: Saline 0 4 ml Pre-exp	Exp	N	Chlorpromazine 1 0 Pre-exp	Exp	N	Chlorpromazine 2 5 Pre-exp	Exp	N
Mount									
Percentage	49	53	57	58	36[b]	50	53	10[c]	30
Latency[Md]	20	15	23	20	10	18	50	30	2
No/min[Md]	2 7	3 0	23	2 5	3 0	18	3 2	2 2	2
Intromission									
Percentag	32	39	57	38	36	50	43	7[b]	30
Latency[Md]	23	23	8	35	45	9	20	10	1
Ratio[Md]	61	61	8	53	67	9	67	82	1
Ejaculation									
Percentage	0	0	57	0	0	50	0	0	30

The Effect of Chlorpromazine

Chlorpromazine 1 0 mg/kg given 1 hour before the copulatory tests caused a slight but significant, decrease in mount percentage (table V) The decrease in mount percentage was more pronounced after chlorpromazine 2 5 mg/kg This dose also decreased intromission percentage significantly No other parameter was significantly changed

The gross appearance was not affected by chlorpromazine 1 0 mg/kg A slight ptosis and reduced locomotor activity was seen after 2 5 mg/kg (see below) Before the introduction of the female into the observation cage the subjects treated with chlorpromazine 2 5 mg/kg had a tendency to stand immobile This tendency was no longer present after the introduction of the female

TABLE VI The effect of pimozide on copulatory behavior in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. Drug and saline treatments were given 3 hours before the experimental test. Md median ^{a} p 0 05 b p 0 01 ^{c} p 0 001

Copulatory paramete	Treatment mg/kg Saline 0 4 ml Pre-exp	Exp	N	Pimo id 0 10 Pre-exp	Exp	N	Pimozide 0 25 Pre-exp	Exp	N
Mount									
Percentag	54	54	41	53	50	40	55	21^c	42
Latency^{Md}	18	25	16	25	18	18	15	25^b	9
No/mi ^{Md}	2 7	2 3	15	2 5	2 7	18	4 0	2 3	9
Intromi ion									
Percent g	41	34	41	38	33	40	45	19	42
Latency^{Md}	23	30	10	30	30	9	20	53	8
Ratio^{Md}	61	50	10	64	38	9	70	52	8
Ej cul tion									
P rcentag	0	2	41	0	0	40	0	0	42

The Effect of Pimozide

Pimozide 0 10 mg/kg given 3 hours before the copulatory tests did not significantly change any of the copulatory parameters but 0 25 mg/kg significantly decreased mount percentage and intromission percentage increased mount latenc and decreased the number of mounts/min (table VI)

In contrast the overt behavior was unaffected: there was no ptosis or reduction in motor activity (see below)

TABLE VII. The effect of phenoxybenzamine on copulatory behavior in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. Drug and saline treatments were given 2 hours before the experimental test. Md = median.

Copulatory parameter	Treatment mg/kg Saline 0 4 ml Pre-exp	Exp	N	Phenoxybenzamine 1 0 Pre-exp	Exp	N	Phenoxybenzamine 3 0 Pre-exp	Exp	N
Mount									
Percentage	53	56	36	43	53	30	57	47	30
Latency Md	15	10	15	15	15	12	15	10	11
No/min Md	3 0	3 3	15	3 3	3 0	12	2 8	3 2	10
Intromission									
Percentage	42	44	36	40	33	30	47	37	30
Latency Md	30	20	13	33	80	8	20	20	8
Ratio Md	67	53	13	66	53	8	77	58	8
Ejaculation									
Percentage	0	0	36	0	0	30	3	3	30

The Effect of Phenoxybenzamine

There was no significant effect on any of the copulatory parameters recorded after phenoxybenzamine 1 0 or 3 0 mg/kg given 2 hours before the copulatory tests (table VII)

The subjects had clearcut ptosis and reduced locomotor activity after phenoxybenzamine 3 0 mg/kg (see below) but not after 1 0 mg/kg

At a still higher dose (10 mg/kg) the subjects were crouched immobile and did not take any notice of the female

TABLE VIII The effect of propranolol on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. Drug and saline treatments were given 45 min. before the experimental test. Md = median. b p 0 01 c p 0 001

Copulatory pa ameter	Treatme t mg/kg Saline 1 6 ml Pre-exp	Exp	N	Propr nolol 1 0 P e-exp	Exp	N	Propranolol 4 0 Pre-exp	Exp	N
Mount									
P centage	48	44	54	44	50	36	56	53	36
Latency[Md]	25	20	19	25	10	13	15	10	16
Mo/min[Md]	2 0	2 3	19	2 7	2 0	13	2 7	1 5[b]	16
Intromission									
Percentage	26	30	54	28	19	36	47	17[c]	36
Latency[Md]	58	50	10	65	25	5	45	30	6
Rati [Md]	54	58	10	60	22	5	57	25	6
Ejacul tion									
Percentage	0	0	54	0	0	36	0	0	36

The Effect of Propranolol

There was no significant change in mount percentage after propranolol 1 0 or 4 0 mg/kg given 45 min before the copulatory tests (table VIII) However propranolol 4 0 mg/kg significantly decreased intromission percentage and the number of mounts/min All subjects given propranolol had a decreased intromission ratio (p < 0 01 pooled data from both dosage groups)

The overt behavior was not influenced by propranolol in the doses used

TABLE IX. The effect of certain drug treatments on motor activity in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. One animal at a time was run for 10 min. in an Animex[R] activity meter beginning at zero hour. Statistical evaluation was performed with the Mann-Whitney U test. p 0.05 b p 0.01, c = p 0.001

MOTOR ACTIVITY

Treatment mg/kg	At hour	Counts/10 min ±	m.	N
Saline 0 4 ml	30	764 + 34		8
Reserpine 0 5	30	521 22[c]		8
Saline 0 4 ml	2	853 ± 36		6
Tetrabena ine 1 0	2	718 + 45[b]		6
T t abena ine 3 0	2	426 ± 67[b]		6
Saline 4 0 4 ml	7# 54 -30 -6	916 34		6
PCPA 4 x 100	7# 54 30 -6	336 ± 62[b]		6
αMT 150	3	401 + 29[b]		6
Saline 0 4 ml	6	760 28		9
αMT 75	6	668 ± 54		9
Saline 0 4 ml	4	871 ± 19		7
FLA-63 10	4	514 ± 23[c]		7
Saline 0 4 ml	1	773 ± 18		10
Chlorpromazine 1 0	1	926 27[c]		10
Chlorpromazine 2 5	- 1	680 + 19[a]		7
Phenoxybenzamine 1 0	2	593 + 25[c]		10
Saline 0 4 ml	3	738 + 27		9
Pimozide 0 25	3	884 ± 27		9
Saline 1 6 ml	0 75	801 ± 56		6
Propranol 1 4 0	0 75	832 ± 44		6

TABLE X. Comparison of the effect of certain drug treatments on mount percentage and motor activity + = increased, decreased. NS = not significant (p 0 05) one symbol = p 0 05 two symbols = p 0 01 three symbols = p 0 001 Statistics mount percentage = difference between pre-experimental and experimental test, motor activity = difference between drug and saline treated subjects

DRUG TREATMENT	MOUNT PERCENTAGE	MOTOR ACTIVITY
Reserpine 0 5 mg/kg		-
Tetr bena ine 1 0 mg/kg		
Tetrabena in 3 0 mg/kg		
PCPA 4 x 100 mg/kg	+ +	
αMT 75 mg/kg		NS
αMT 150 mg/kg		
FLA-63 10 mg/kg	NS	
Chlorproma ine 1 0 mg/kg		+
Chlorproma ine 2 5 mg/kg		
Pimozide 0 25 mg/kg		NS
Phenoxybenzamine 3 0 mg/kg	NS	
Propr nolol 4 0 mg/kg	NS	NS

Motor Activity

Motor activity as measured by the Animex[R] activity meter was significantly increased by chlorpromazine 1 0 mg/kg and not significantly changed by αMT 75 mg/kg pimozide 0 25 mg/kg and propranolol 4 mg/kg (table IX) Motor activity was decreased by all other drugs and doses tested (reserpine 0 5 mg/kg tetrabenazine 1 0 and 3 0 mg/kg PCPA 4 x 100 mg/kg αMT 150 mg/kg FLA-63 10 mg/kg chlorpromazine 2 5 mg/kg and phenoxybenzamine 3 0 mg/kg) For comparison of the effect of the drugs on mount percentage and motor activity table X was constructed It is apparent that a change

in one behavior is not necessarily associated with a corresponding change in the other behavior It is of particular interest that the facilitatory effects of PCPA can occur in spite of a pronounced decrease in motor activity

Scoring of the Orientation of Activity during Copulatory Tests

Each subject was given the score of 1 for each of the 3 min in which a certain orientation of activity was displayed for at least 0 05 min consecutively The following classes of behavior were distinguished: Activity oriented 1 towards the female 2 towards the environment 3 towards the subject itself and 4 immobility The distribution of pre-experimental scores is given in table XI and the changes of scores after treatments are given in tables XII (reserpine and tetrabenazine) XIII (αMT and FLA-63) XIV (chlorpromazine and pimozide) and XV (phenoxybenzamine and propranolol)

Irrespective of subsequent mounting or non-mounting female-oriented activity (enough to be scored) was displayed by all subjects during the first min of the test Thus the change of scores for female-oriented activity only reflects the change obtained during the last two of the 3 min During these two min. the scores for female-oriented activity were significantly decreased among pimozide 0 25 mg/kg treated non-mounters The scores for environment-oriented activity were significantly increased among αMT 75 mg/kg treated non-mounters and pimozide 0 25 mg/kg treated mounters and decreased among reserpine 0 5 mg/kg treated non-mounters The scores for self-oriented activity were increased among chlorpromazine 1 0 mg/kg treated mounters Tetrabenazine 3 0 mg/kg treated non-mounters received increased scores for immobility No other significant change of the scores was obtained. Thus a general feature of the compounds tested is that a dose sufficient to change the copulatory behavior has fairly small effects on the other behavior patterns recorded

TABLE XI Orientation of activity during copulatory tests in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The table shows the percentage distribution of p[illegible] [illegible] scores Each subject was given the score of 1 for each of the 3 min in which certain behavior was displayed for at least 0 05 min, consecutively

Percentage distribution of p e-experimental scores

	Orientation toward															
	femal				environment				self				Immobility			
Sco	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Mounters (N 281)	0	2	19	80	18	37	36	10	2	13	29	56	99	1	0	0
Non-mounters (N = 319)	0	59	30	11	0	8	51	41	22	39	33	6	100	0	0	0

TABLE XII Orientation of activity during copulatory tests The table shows the number of subjects which, after treatment with saline or reserpine (A) resp. tetrabenazine (B) (exp. test) obtained score different from the one in the pre-exp 1 test. Scoring technique: see table XI and Methods.

Treatment (mg/kg)	Orientation towards: female			environment			self			Immobility		
	Direction of changed score in the exp test and the associated probability											
	−	+	p	−	+	p	−	+	p	−	+	p
A1 Saline 0 4 ml												
mounters (N 11)	0	1	NS	4	2	NS	3	1	NS	0	0	NS
non-mounters (N 12)	4	2	NS	4	2	NS	2	5	NS	0	0	NS
A2 Reserpine 0 5												
mounters (N 11)	2	1	NS	5	3	NS	5	0	NS	0	0	NS
non-mounters (N 16)	5	5	NS	11	1	0 01	3	5	NS	0	1	NS
B1 Saline 0 4 ml												
mounters (N 14)	3	1	NS	4	4	NS	2	3	NS	1	0	NS
non-mounters (N = 13)	5	3	NS	4	6	NS	3	4	NS	0	0	NS
B2 Tetrabenazine 1 0												
mounter (N = 16)	5	2	NS	4	8	NS	6	5	NS	0	2	NS
non-mounter (N 21)	8	6	NS	4	8	NS	5	6	NS	0	0	NS
B3 Tetrabenazine 3 0												
mounter (N 4)	0	0	NS	0	2	NS	1	0	NS	0	1	NS
non-mounter (N = 19)	2	5	NS	3	2	NS	11	4	NS	0	10	0 001

TABLE XIII Orientation of activity during copulatory tests The table shows the number of subjects which after treatment with saline or αMT (A) resp. FLA-63 (B) (exp. test) obtained score different from the one in the pre-experimental test. Scoring technique: see table XI and Methods

Orientation towards

Direction of changed score in the exp t st and th associated probability

T eatment (mg/kg)	female			environment			self			Immobility		
			p			p			p			p
A1 Saline 0 4 ml												
mounter (N 14)	0	3	NS	4	2	NS	2	2	NS	0	0	NS
non-mou te (N 6)	1	2	NS	3	3	NS	2	3	NS	0	0	NS
A2 MT 75												
mounter (N 13)	7	1	NS	2	9	NS	6	2	NS	0	0	NS
non-mounters (N 14)	3	2	NS	0	6	0 05	4	6	NS	0	0	NS
B1 Saline 0 4 ml												
mounter (N 15)	4	3	NS	6	4	NS	4	4	NS	0	0	NS
non-mounters (N 14)	3	3	NS	6	3	NS	3	4	NS	0	0	NS
B2 FLA-63 10												
mounters (N 13)	1	4	NS	4	4	NS	1	2	NS	0	1	NS
non-mounter (N 13)	3	5	NS	7	1	NS	6	4	NS	0	1	NS

TABLE XIV Orientation of activity during copulatory tests The table shows the number of subjects which, after treatment with saline or chlorpromazine (A) resp. pimozide (B) (exp test) obtained score different from the one in the pre-experimental test. Scoring technique see table XI and Methods

Orientation towards

Direction f hanged score i the xp t st nd the ssociated probability

	Treatment (mg/kg)	female –	female +	female P	environment –	environment +	environment P	self –	self +	self P	Immobility –	Immobility +	Immobility P
A1	Saline 0 4 ml												
	mounter (N 23)	3	5	NS	7	8	NS	6	9	NS	0	0	NS
	non-mounters (N = 22)	6	5	NS	5	8	NS	5	7	NS	0	0	NS
A2	Chlorpromazine 1 0												
	mounters (N 18)	2	2	NS	9	2	NS	1	8	0 05	0	1	NS
	non-mount rs (N 20)	4	6	NS	6	11	NS	6	9	NS	0	0	NS
A3	Chlorpromazine 2 5												
	mounter (N 2)	1	0	NS	1	0	NS	1	0	NS	0	0	NS
	non-mounter (N = 13)	2	2	NS	2	7	NS	3	5	NS	0	0	NS
B1	Saline 0 4 ml												
	mounters (N 15)	3	2	NS	5	6	NS	4	6	NS	0	0	NS
	non-mounters (N 13)	1	3	NS	1	3	NS	4	3	NS	0	0	NS
B2	Pimozid 0 10												
	mounters (N 18)	3	1	NS	7	7	NS	5	6	NS	0	0	NS
	non-mounters (N 17)	6	3	NS	2	6	NS	7	6	NS	0	0	NS
B3	Pimozide 0 25												
	mounters (N 9)	2	0	NS	0	7	0 05	4	0	NS	0	0	NS
	non-mounter (N 19)	6	1	0 05	4	5	NS	3	11	NS	0	0	NS

TABLE XV Orientation of activity during copulatory tests The table shows the number of subjects which after treatment with saline or phenoxybenzamine (A) resp. propranolol (B) obtained score different from the one in the pre-experimental test. Scoring technique: see table XI and Methods

Orientation toward

Di ection of changed sco i the xp test and the sociated probability

Treatment (mg/kg)	femal			environme t			self			Immobility		
			p		+	p			p		+	p
A1 Saline 0 4 ml												
mounter (N = 15)	2	1	NS	2	5	NS	2	3	NS	0	0	NS
non-mounters (N 12)	6	3	NS	4	4	NS	3	5	NS	0	0	NS
A2 Phenoxybenzamine, 1 0												
mount rs (N 12)	2	1	NS	5	4	NS	5	0	NS	0	1	NS
non-mounters (N = 13)	3	3	NS	2	3	NS	4	2	NS	0	1	NS
A3 Phenoxybenzamine, 3 0												
mounter (N = 10)	1	1	NS	3	2	NS	3	1	NS	0	3	NS
non-mounters (N 10)	2	2	NS	3	2	NS	2	5	NS	0	2	NS
B1 Saline 1 6 ml												
mount rs (N 19)	4	7	NS	5	6	NS	7	5	NS	0	0	NS
non-mounter (N 22)	3	3	NS	5	5	NS	4	6	NS	0	0	NS
B2 Propranol 1 1 0												
mounter (N 13)	5	3	NS	4	4	NS	4	1	NS	0	0	NS
non-mounter (N = 15)	4	3	NS	4	4	NS	2	6	NS	0	0	NS
B3 Propranol 1 4 0												
mounter (N 16)	5	1	NS	4	6	NS	7	2	NS	0	0	NS
non-mounters (N 13)	1	2	NS	1	4	NS	4	6	NS	0	0	NS

DISCUSSION

The two monoamine depletors reserpine and tetrabenazine suppressed testosterone-activated heterosexual behavior in the castrated male rat The specificity of this effect is suggested by the fact that the non-copulatory female-oriented activity which requires intact motor capabilities was not decreased Abolishment of copulatory behavior in intact male rats by tetrabenazine (5 mg/kg) has previously been reported by Butcher et al (1969) From the description of the overt behavior of their subjects it is obvious that they lacked the motor capabilities necessary for copulatory behavior to be displayed. Facilitatory effects on certain components of male copulatory behavior of reserpine (Soulairac 1963; Dewsbury and Davis 1970) and tetrabenazine (Dewsbury 1972) have also been reported Since intact rats were used in these studies and other parameters recorded direct comparison with the present data cannot be made

After repeated PCPA treatment there was an increased mount and intromission percentage a decreased mount latency an increased number of mounts/min. and an increased ejaculation percentage With the exception of ejaculation percentage the same effects were obtained both in non-adrenalectomized and adrenalectomized subjects Thus these effects on copulatory behavior were not secondary to an altered endogenous steroid hormone production

Contrary to the effects of PCPA on the copulatory behavior αMT had clearcut inhibitory effects: the mount and intromission percentages were decreased the mount latency increased and the number of mounts/min. decreased Notably αMT 75 mg/kg did not significantly reduce motor activity while a pronounced decrease of motor activity was obtained by the repeated PCPA treatment.

When the data on selective serotonin (PCPA) and catecholamine (αMT) synthesis inhibition are compared it is obvious that the effects were opposite to each other with respect to mount and intromission percentages mount latency and the number of mounts/min.

Since catecholamine but not serotonin depletion was inhibitory these data suggest that reserpine and tetrabenazine inhibited components of the male copulatory behavior mainly by impairing catecholaminergic neurotransmission.

FLA-63 10 mg/kg decreased motor activity but copulatory behavior was not inhibited In contrast to the decreased number of mounts/min after αMT 75 mg/kg FLA-63 increased the number of mounts/min The difference in effect between αMT and FLA-63 indicates the significance of dopamine rather than noradrenaline for male copulatory behavior This is further suggested by the effects of the receptor blocking agents

Pimozide which in the doses used blocks dopamine but not noradrenaline receptors brought about inhibitory effects in a dose which did not decrease motor activity or otherwise disturb the overt behavior: mount and intromission percentages were decreased and there was an increased mount latency and a decreased number of mounts/min Furthermore the scores for female-oriented activity were significantly decreased among pimozide treated non-mounters This finding indicates that pimozide not only inhibits copulatory behavior but also other components of the male s interaction with the female

Phenoxybenzamine which is considered to block noradrenaline receptors but not dopamine receptors centrally and α-receptors peripherally decreased motor activity but did not inhibit copulatory behavior In contrast chlorpromazine which blocks both noradrenaline and dopamine receptors induced a decrease of the mount and intromission percentages

Propranolol which blocks adrenergic β-receptors did not decrease mount percentage but decreased intromission percentage intromission ratio and the number of mounts/min Further experimentation is needed to elucidate the relationship between these effects and a blockade of adrenergic β-receptors Notably propranolol was the only compound tested which changed intromission percentage without affecting mount percentage

The data of the present investigation show that decreased catecholaminergic tone inhibits copulatory behavior in the male rat. Provided the noradrenergic tone was not more than moderately decreased no inhibition of the behavior was brought about; certain components were even facilitated (such as an increased number of mounts/min after FLA-63) If the noradrenergic tone was decreased to the extent that there were profound effects on the overt behavior copulatory behavior was inhibited In contrast a drug-induced decrease of the dopaminergic tone inhibited copulatory behavior already in doses which produced little or no alteration of the overt behavior

In methodologically analogous experiments it was recently found in this laboratory that an increase of the central nervous catecholamine content by means of L-DOPA, given after a combined treatment with a monoamine oxidase inhibitor (pargyline) and an extracerebral decarboxylase inhibitor (MK486) facilitated several components of the copulatory behavior while an increase of the serotonin content by means of addition of DL-5-HTP had essentially the opposite effect (Malmnäs and Meyerson 1972; Malmnäs 1973b) No facilitation of copulatory behavior was obtained by the selective noradrenaline precursor DL-DOPS When considering the results of the present investigation in relation to the ones with monoamine precursors it appears that a decreased central nervous serotoninergic tone facilitates a decreased dopaminergic tone inhibits while a decreased noradrenergic tone has no specific inhibitory effect on [illegible] activated heterosexual copulatory behavior in the castrated male rat

For gener us suppli of hormones and drugs I thank Organon, the Neth rlands through Er Sweden (test sterone propionate estradi l benzoat prog terone and fre dexamethasone) Dr H Corrodi Hä 1 Sweden (dl-p-chlorophenylalanine methyle t HCl dl-α-methyl-p-tyr sine methyl ter HCl FLA-63) Ciba Switz rl nd (re s rpine) Hoffmann-La Roche Switz rland (t tr benazine HCl) Leo Swed n (chlorpromazine HCl) Jans n Belgium (pimozide) Smith Kline & French England (phenoxybenzamine HCl) and ICI England

through Scanmeda Sweden (dl-propranolol HCl) The work was supported by NIH grant R01- HD04108-03 and the Swedish Medical Research Council grant 14X-64-08 to Dr Bengt Meyerson and grants from the Medical Faculty University of Uppsala to the author

REFERENCES

AHLENIUS S H ERIKSSON K LARSSON K MODIGH and P SÖDERSTEN Mating behavior in the male rat treated with p-chlorophenylalanine methyl ester alone and in combination with pargyline Psychopharmacologia (Berl) 1971 20 383-388

ANDÉN N E S G BUTCHER H CORRODI K FUXE and U UNGERSTEDT Receptor activity and turnover of dopamine and noradrenaline after neuroleptics European J Pharmacol 1970 11 303-314

ANDÉN N E H CORRODI K FUXE and T HÖKFELT Increased impulse flow in bulbospinal noradrenaline neurons produced by catecholamine receptor blocking agents European J Pharmacol 1967 2 59-64

ANDÉN N E A DAHLSTRÖM K FUXE and K LARSSON Functional role of the nigro-neostriatal dopamine neurons Acta Pharmacol (Kbh) 1966a 24 263-274

ANDÉN N E H G M JUKES and A LUNDBERG The effect of DOPA on the spinal cord 2 A pharmacological analysis Acta physiol scand 1966 67 387-397

BLACK J W A F CROWTHER R G SHANKS L H SMITH and A C DORNHORST A new adr nergic beta receptor antagonist The Lancet 1964 1 1080-1081

BOYD V J E E SHILLITO and M VOGT Influence of age nd of testosterone on the response of male rats to parachlorophenylalanine Brit J Pharmacol 1972 46 46-55

BUTCHER L L S G BUTCHER and K LARSSON Effects of apomorphine (+)-amphetamine and nialamide on tetrabenazine-induced suppression of sexual behavior in the male rat European J Pharmacol 1969 7 283-288

DEWSBURY D A Effects of tetrabenazine on the copulatory behavior of male rats European J Pharmacol 1972 17 221-226

DEWSBURY D A and H N DAVIS Effects of reserpine on the copulabory behavior of male rats Physiol Behav 1970 5 1331-1333

FLORVALL L and H CORRODI Dopamine β-hydroxylase inhibitors Acta Pharm Suecia 1970 7 7-22

GESSA G L A TAGLIAMONTE P TAGLIAMONTE and B B BRODIE Essential role of testost rone in the sexual stimulation induced by p-chlorophenylalanine in male animals Nature (Lond) 1970 227 616-617

JEQUIER E W LOVENBERG and A SJOERDSMA Tryptophan hydroxylase inhibition: the mechanism by which p-chlorophenylalanine depletes rat brain serotonin Mol Pharmacol 1967 3 274 278

KOE B K and A WEISSMAN p-Chlorophenylalanine: a specific depletor of brain serotonin J Pharmacol Exp Ther 1966 154 499–516

MALMNÄS C O Testosterone-activated copulatory behavior in the castrated male rat Acta physiol scand 1973a Suppl 395 9–46

MALMNÄS C O Monoamine precursors and copulatory behavior in the male rat Acta physiol scand 1973b Suppl 395 47–68

MALMNÄS C O and B J MEYERSON p-Chlorophenylalanine and copul tory behaviour in the male rat Nature (Lond) 1971 232 398–400

MALMNÄS C O and B J MEYERSON *Monoamines and copulatory activation in the castrated male rat* Acta Pharmacol et Toxicol 1972 31 Suppl 1 23

MITLER M M B MORDEN S LEVINE and W DEMENT Th effects of par chloroph nylalanine on the mating behavior in male rats Physiol Behav 1972 6 1147–1150

SALIS P J and D A DEWSBURY p-Chlorophenylalaninen facilitates copulat ry behaviour in male rats Nature (Lond) 1971 232 400–401

SHEARD M H The eff ct of p-chlorophenylalanine on behavior in rats: relation to brain erotonin and 5-hydroxyindoleacetic cid Brain Research 1969 15 524-528

SHILLITO E E The ffect of parachlorophenylalanine on soci l interactions of male rats Brit J Pharmacol 1970 38 305–315

SIEGEL S *Nonparametric statistics for the behavioral sciences* (McGraw Hill New York 1956)

SOULAIRAC M L Étude expérimental des régulations hormono-nerveuse du comportement sexuel du Rat mâl Ann d Endocr 1963 24 Suppl to N 3 1–98

SPECTOR S A SJOERDSMA and S UDENFRIEND Blockade of endogenous norepinephrine ynthe i by α-methyl-tyro ine an inhibitor of tyrosine hyd xylas J Pharmacol Exp Ther 1965 147 86–95

TAGLIAMONTE A P TAGLIAMONTE and G L GESSA Reversal f pargyline-induced inhibition f sexual behaviour in male rats by p-chlorophenylalanine Nature (Lond) 1971 230 244–245

TAGLIAMONTE A P TAGLIAMONTE G L GESSA nd B B BRODIE Compulsive xual ctivity induced by p-chl rophenylalanin in normal and pinealectomi ed male r ts Science 1969 166 1433–1435

From the Department of Medical Pharmacology
University of Uppsala Uppsala Sweden

EFFECTS OF LSD-25 CLONIDINE AND APOMORPHINE ON COPULATORY BEHAVIOR IN THE MALE RAT

By

CARL OLOF MALMNÄS

ABSTRACT

The effects of lysergic acid diethylamide (LSD) clonidine and apomorphine on testosterone activated heterosexual copulatory behavior in castrated male rats were studied Th perc ntage of subjects which displayed copulatory behavior was decreased by LSD 30 and 100 µg/kg while this percentage was increased after apomorphine 30 100 and 300 µg/kg The facilitatory effects of apomorphine 100 µg/kg were blocked by pimozide 100 µg/kg Clonidine 3 10 and 30 µg/kg did not change the percentage of subjects that displayed copulatory behavior The results are discussed in terms of the possible relation to monoaminergic mechanisms involved in the regulation of certain components of male sexual b havior

INTRODUCTION

Accumulating evidence suggests that lysergic acid diethylamide (LSD) mimics and/or facilitates the effect of serotonin on central nervous serotoninergic receptors (Freedman 1961; Curtis and Davis 1962; Rosecrans et al 1967; Andén et al 1968; Diaz et al 1968; Aghajanian et al 1968 1970 1972; Lin et al 1969; Schubert et al 1970; Eliasson et al 1972; for review see Aghajanian 1972a b) There is also evidence that clonidine has noradrenaline-like effects in the central nervous system (Andén et al 1970; Schmitt et al 1971; Broekkamp and van Rossum 1972) Furthermore

increasing evidence indicates that apomorphine mimics and/or facilitates the effects of dopamine in the central nervous system (Ernst 1967; Andén et al 1967; Roos 1969; Ungerstedt et al 1969; Ungerstedt 1971)

This investigation deals with the effects of LSD clonidine and apomorphine on testosterone-activated copulatory behavior in the castrated male rat These drugs were used as tools in order to further elucidate the neural mechanisms involved in the regulation of this behavior

MATERIAL AND METHODS

A proc dure has been developed to maintain castrated male rats on a submaximal respons l v l with r spect to th p rcentage of subjects which displayed copulatory behavior (Malmnäs 1973a) The method is also described in detail in Malmnäs (1973b) from which no change ha been made (See page 48 in this supplement)

Subjects

Experimental subjects were about 195 male Wistar ats castrated as adult

Injected materials

Th hormones us d wer te t teron propionat (TP) estradiol benzoat and proge t ne (Organon) Th neuropharmacological compounds used were D-lys rgi acid di thylamid tartrat (LSD Sand z Basel) cl nidine HCl (CH Boehringer Sohn Ingelheim am Rhein) apomorphine HCl (Sando Bas l) and pimozide (Janssen Be s) The hormone were di olved in olive oil and the neuropharmacological agents (xcept pimozide) in saline; pimo ide was dissolved in few drop f gl ci l acetic acid dilut d with saline and the pH adjust d to 5 by ddition f 2 N NaOH All inj ctions f hormones we given ubcutaneously whil the neuropharmalogical gents and blank solution were given intrap ritoneally all in a volume f l ml/kg Do mentioned in th text and tables alway refe t th f rms f th compounds tat d above

Th time from injection of LSD clonidine and apomorphine to th ons t f the copulatory t sts we ba ed on pilot xp riments in which r tings of th overt behavior were made every six min. from inj ction

RESULTS

The Effect of LSD

LSD 10 30 or 100 µg/kg was given 12 min before the onset of the copulatory tests The time 12 min was based on pilot experiments which showed that the effects on the overt behavior of LSD 300 µg/kg (side-movements of the head hyperextensions of hindlegs tremor) had reached its maximum at that time

LSD 10 µg/kg did not significantly change any of the copulatory parameters recorded (table I) After 30 and 100 µg/kg of LSD mount and intromission percentages were significantly decreased. After 30 µg/kg of the drug there was also a significant decrease of the number of mounts/min. This effect was not possible to evaluate after 100 µg/kg since too few subjects displayed mounting in the exp test. No other copulatory parameters recorded were significantly changed

The overt behavior was unaffected by the two lower doses of LSD tested A few subjects showed side-movements of the head and intermittent hyperextensions of the hindlegs after LSD 100 µg/kg

The Effect of Clonidine

Clonidine 3 10 or 30 µg/kg was given 12 min before the onset of the copulatory tests Pilot experiments showed that pronounced sedation with immobility was fully developed at that time after clonidine 100 µg/kg

There was no significant change of the copulatory parameters recorded after clonidine 3 or 10 µg/kg (table II) Clonidine 30 µg/kg however significantly decreased mount latency as well as the number of mounts/min. No other copulatory parameter was significantly changed.

The overt behavior was not affected by clonidine 3 µg/kg Locomotor activity was slightly decreased after 10 µg/kg and there

TABLE I The effect of LSD on copulatory behavior in castrated male rats maintained on a submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline treatments were given 12 min. before the experimental tests Md median. a = p 0 05 b = p 0 01 c p 0.001

Copulatory parameter	Treatment µg/kg Saline 0 4 ml			LSD 10			LSD 30			LSD 100		
	Pre-exp	Exp	N	Pre-exp	Exp	N	P e-exp	Exp	N	Pre-exp	Exp	N
Mount												
Percentage	53	47	30	44	41	39	56	28[b]	36	63	13[c]	24
Latency Md	15	15	13	20	15	14	20	18	10	10	10	3
No/mi Md	2 7	2 0	13	3 0	2 7	14	3 2	1 0	10	2 0	2 0	3
I tromission												
Percentage	40	30	30	31	23	39	42	8[b]	36	54	4[c]	24
Latency Md	25	25	7	90	30	8	18	33	2	15	15	1
Ratio Md	57	64	7	45	48	8	63	70	2	76	70	1
Ejacul tion												
Percentage	3	3	30	0	0	39	0	0	36	4	0	24

TABLE II. The effect of clonidine on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment The drug and saline treatments were given 15 min before the experimental tests Md median. * p 0 05

Copul tory parameter	Treatment µg/kg S line 0 4 ml P e-exp	 Exp	 N	 Cl nidi 3 Pre-exp	 Exp	 N	 Clonidine 10 Pre-exp	 Exp	 N	 Clonidine 30 Pre-exp	 Exp	 N
Mount												
Percentage	53	57	30	53	43	30	45	45	31	52	45	29
Latency^{Md}	15	10	13	10	10	11	10	10	11	18	10*	10
No/min^{Md}	2 2	2 3	12	2 0	2 7	10	1 7	1 3	11	2 0	1 0	9
I tromission												
Percentage	37	40	30	33	17	30	29	23	31	24	34	29
Latency^{Md}	20	25	7	30	13	2	23	30	4	15	10	5
Ratio^{Md}	57	35	7	35	59	2	42	45	4	60	75	5
Ejacul tion												
Percentage	0	3	30	0	3	30	0	0	31	3	0	29

TABLE III. The effect of apomorphine on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. The drug and saline treatments were given 12 min. before the experimental tests Md median. a p 0 05 b p 0 01 c p 0 001

Copulatory parameter	Treatment µg/kg Saline 0 4 ml Pre-exp	Exp	N	Apomorphine 10 Pre-exp	Exp	N	Apomorphine 30 Pre-exp	Exp	N	Apomorphine 100 Pre-exp	Exp	N	Apomorphine 300 Pre-exp	Exp	N
Mount															
Percentage	45	48	44	47	53	45	52	75[b]	49	48	79	42	46	69[a]	39
Latency[Md]	15	15	17	10	15	15	20	10[b]	23	20	10[b]	20	13	10	16
No/min[Md]	2 0	2 0	17	3 0	2 3	13	2 5	3 7[b]	22	2 3	3 3	19	2 7	2 0[a]	15
Intromission															
Percentage	25	36	44	36	36	45	35	50	48	33	62[b]	42	33	38	39
Latency[Md]	30	40	9	25	28	10	35	25	13	40	20	11	105	35	7
Ratio[Md]	45	40	9	63	70	10	50	58	13	50	50	11	30	56	7
Ejaculation															
Percentage	0	0	44	4	2	45	0	2	48	2	2	42	0	3	39

TABLE IV The effect of pimozide and pimozide apomorphine on copulatory behavior in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment Pimozide was given 3 hours apomorphine 12 min and saline 3 hours (A1) resp. 3 hours 12 min. (B1) before the experimental tests Md median

Copulatory parameter	Treatment µg/kg — A1 Saline 0 4 1			A2 Pimozide 100			B1 Saline 0 4 ml			B2 Pimozide 100 Apomorphine 100		
	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N	Pre-exp	Exp	N
Mount												
Percentage	54	54	41	53	50	40	46	54	39	50	53	36
Latency^{Md}	18	25	16	25	18	18	15	20	15	15	18	16
No/min^{Md}	2 7	2 3	15	2 5	2 7	18	3 0	2 7	14	2 3	2 2	14
Intromission												
Percentage	41	34	41	38	33	40	28	26	39	31	31	36
Latency^{Md}	23	30	10	30	30	9	18	40	6	25	35	8
Ratio^{Md}	61	50	10	64	38	9	66	69	6	68	50	8
Ejaculation												
Percentage	0	2	41	0	0	40	0	3	39	6	0	36

was a more pronounced decrease after clonidine 30 µg/kg At higher doses (100 µg/kg) the subjects sat immobile and did not take any notice of the female

The Effect of Apomorphine

Apomorphine 10 30 100 or 300 µg/kg was given 12 min before the onset of the copulatory tests According to pilot experiments stereotyped gnawing sniffing and licking were fully developed at 12 min. after apomorphine 100 µg/kg

Mount percentage was significantly increased after apomorphine 30 100 and 300 µg/kg (table III) After apomorphine 100 µg/kg there was also a significant increase in intromission percentage Mount latency was significantly decreased after apomorphine 30 and 100 µg/kg The number of mounts/min was not changed by apomorphine 10 µg/kg significantly increased by apomorphine 30 µg/kg not significantly changed by apomorphine 100 µg/kg and significantly decreased by apomorphine 300 µg/kg The decline of the number of mounts/min. with increasing dose of apomorphine was possibly due to the increase of starotyped motor patterns elicited by the higher doses

All subjects treated with apomorphine 300 µg/kg showed some kind of stereotyped behavior ranging from gentle sniffing of the floor to compulsive licking gnawing chewing of wooden shavings from the floor and scratching on the walls of the observation cage Sniffing of the floor and environment was the only stereotyped behavior displayed by subjects treated with apomorphine 100 µg/kg No obvious stereotypies were seen after apomorphine 30 or 10 µg/kg

The Effect of Apomorphine after Pimozide Pretreatment

Pimozide is a neuroleptic which blocks dopamine but not noradrena-

line receptors (Andén et al 1970a) This drug (0 25 mg/kg) has been found to inhibit copulatory behavior as measured by the method used in this investigation (Malmnäs 1973c) In the present experiment pimozide was given in a dose found to have to detectable effects on the copulatory parameters recorded

Pimozide 100 µg/kg was given 3 hours and apomorphine 100 µg/kg 12 min before the copulatory tests All the effects on the copulatory behavior obtained by apomorphine 100 µg/kg were prevented by pimozide 100 µg/kg (table IVB)

The overt behavior was unaffected by this treatment regimen. No subject showed any stereotyped sniffing of the floor or environment as was seen when this dose of apomorphine was given alone

Motor Activity

Motor activity as measured by the Animex[R] activity meter was decreased by clonidine 30 µg/kg and by apomorphine 100 µg/kg while it was not significantly changed by LSD 30 µg/kg (table V) Pretreatment with pimozide 100 µg/kg blocked the apomorphine induced decrease in motor activity For comparison between the effect of certain drug treatments on mount percentage and motor activity table VI was constructed It is apparent that a change in one behavior is not associated with a corresponding change in the other behavior

Scoring of the Orientation of Activity during Copulatory Tests

Each subject was given the score of 1 for each of the 3 min. in which a certain orientation of activity was displayed for at least 0 05 min consecutively The following classes of behavior were distinguished: Activity oriented 1 towards the female 2 towards the environment 3 towards the subject itself and 4 immobility The results are given in tables VII (pre-exp scores) VIII (LSD)

TABLE V The effect of certain drug treatments on motor activity in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment. One animal at time was run for 6 min. in an Animex[R] activity meter starting t zero min Statistical evaluation was performed with the Mann-Whitney U test.

MOTOR ACTIVITY

	Treatme t µg/kg	At min	Counts/6 mi	s e m	N
A1	Saline 0 4 ml	12	529	23	6
A2	LSD 30	12	544	27	6
A3	Clonidine 30	12	374	30	6
A4	Apomorphine 100	- 12	426 ±	28	6
B1	Saline 0 4 ml	180 - 12	491 ±	18	6
B2	Pimozid 100	180			
	Saline 0 4 ml	12	502	20	6
B3	Pimozid 100	180			
	Apomorphine 100	12	476 ±	31	6

Probabiliti p _ soci_ted with the _co es of the diff_re t tre tment groups

Groups compared	p	Groups compared	p
A1 and A2	NS	B1 and B2	NS
A1 and A3	0 001	B1 and B3	NS
A1 and A4	0 001	B2 and B3	NS
A2 and A3	0 01		
A2 and A4	0 05		
A3 and A4	NS		

TABLE VI Comparison between the effect of certain drug treatments on mount percentage and motor activity increased, - decreased. NS not significant (p 0 05) one symbol p 0 05 two symbols = p 0 01, three symbols p 0 001. Statistics mount percentage = difference between pre-experimental and experimental test, motor activity difference between drug and saline treated subjects

DRUG TREATMENT	MOUNT PERCENTAGE	MOTOR ACTIVITY
LSD 30 µg/kg		NS
Clonidine 30 µg/kg	NS	
Apomorphine 100 µg/kg	+ +	
Pimozid 100 µg/kg	NS	NS
Pimozide 100 µg/kg + Apomorphine 100 µg/kg	NS	NS

TABLE VII Orientation of activity during copulatory tests in castrated male rats maintained on submaximal response level with respect to mount percentage by means of weekly testosterone propionate treatment The table shows the percentage distribution of pre-experimental scores Each subject was given the score of 1 for each of the 3 min. in which certain behavior was displayed for at least 0 05 min consecutively

Pe centage di tribution f pre-experimental scores

	Orientation towards															
	femal				nvironment				self				Immobility			
Score	0	1	2	3	0	1	2	3	0	1	2	3	0	1	2	3
Mounters (N 229)	0	5	23	72	21	38	28	14	5	23	28	44	100	0	0	0
Non-mounter (N 225)	0	56	33	11	1	11	52	36	20	36	37	8	100	0	0	0

TABLE VIII The effect of LSD on orientation of activity during copulatory tests The table shows the number of subjects which in the experimental test received score different from the one in the pre-experimental test. Scoring technique see table VII and Methods.

	Orientation towards											
	femal			environment			self			Immobility		
	Direction of changed sco			in the exp			test and the			associated probability		
Treatment (µg/kg)			p			p		+	p		+	p
Saline 0 4 ml												
mounters (N 13)	3	0	NS	3	4	NS	3	2	NS	0	0	NS
non-mounters (N 13)	5	4	NS	2	4	NS	3	3	NS	0	0	NS
LSD 10												
mounters (N 14)	2	2	NS	5	4	NS	5	3	NS	0	0	NS
non-mounter (N 20)	4	5	NS	5	4	NS	6	8	NS	0	0	NS
LSD 30												
mounter (N 10)	4	1	NS	1	7	NS	6	1	NS	0	0	NS
non-mounters (N 16)	8	0	0 01	2	8	NS	9	0	0 01	0	0	NS
LSD 100												
mounters (N = 3)	1	1	NS	1	1	NS	1	0	NS	0	2	NS
non-mounters (N 9)	3	0	NS	0	7	0 05	4	1	NS	0	1	NS

Table XI The effect of pimozide and pimozide apomorphine on orientation of activity during copulatory tests The table shows the number of subjects which in the experimental test received score different from the one in the pre-experimental test Scoring technique: see table VII and Methods

	Orientation toward									Immobility		
	female			environment			self					
	Direction of changed score in the exp test and the associated probability:											
Treatment (µg/kg)			p		+	p		+	p		+	p
A1 Saline 0 4 ml												
mounters (N 15)	3	2	NS	5	6	NS	4	6	NS	0	0	NS
non-mounter (N 13)	1	3	NS	1	3	NS	4	3	NS	0	0	NS
A2 Pimozide 100												
mounter (N 18)	3	1	NS	7	7	NS	5	4	NS	0	0	NS
non-mount r (N 17)	6	3	NS	2	6	NS	7	6	NS	0	0	NS
B1 Saline 0 4 ml												
mounters (N 14)	2	4	NS	2	3	NS	6	4	NS	0	0	NS
non-mounter (N 15)	4	2	NS	3	5	NS	9	5	NS	0	0	NS
B2 Pimo ide 100 Apomorphine 100												
mounters (N 14)	4	2	NS	5	6	NS	6	3	NS	0	0	NS
non-mounters (N 15)	3	6	NS	5	1	NS	6	7	NS	0	0	NS

IX (clonidine) X (apomorphine) and [illegible]

A certain amount of female-oriented [illegible] was displayed by all subjects during the [illegible] change of scores for female-oriented [illegible] change obtained during the last two of the [illegible] two min. the scores for female-oriented [illegible] among LSD 30 µg/kg treated non-mounters and [illegible]phine 30 and 100 µg/kg treated non-mounters [illegible] morphine 30 µg/kg treated mounters LSD [illegible] mounters got increased scores for environment[illegible] while apomorphine 30 µg/kg treated mounters [illegible] for this orientation of activity Sol[illegible] creased among LSD 30 µg/kg treated non[illegible] immobility were increased in clonidine [illegible] as well as among apomorphine 300 µg/kg [illegible] mounters No other statistically signif[illegible] for the different orientations of activity [illegible]

DISCUSSION

The results of the present investigation [illegible] and apomorphine had effects on testosterone[illegible] copulatory behavior in the castrated ma[illegible] affected copulatory behavior in differen[illegible] probable mechanisms of action of these [illegible] the results will be discussed in relatio[illegible] been found in methodologically analogous [illegible] fects on copulatory behavior of other dr[illegible] gic neurotransmission (cf Malmnäs and [illegible] 1973b c)

Table XI The effect of pimozide and pimozide + apomorphine on orientation of activity during copulatory tests. The table shows the number of subjects which in the experimental test recorded a score different from the one in the pre-experimental test. Scoring technique see table VII and Methods

		Orientation towards											
		female			environment			self			Immobility		
		Direction of changed sco e in th exp test and the associ ted probability											
	Treatme t (μg/kg)			p			p			p		+	p
A1	Saline 0 4 ml												
	mounters (N 15)	3	2	NS	5	6	NS	4	6	NS	0	0	NS
	non-mount r (N 13)	1	3	NS	1	3	NS	4	3	NS	0	0	NS
A2	Pimozid 100												
	mounters (N 18)	3	1	NS	7	7	NS	5	6	NS	0	0	NS
	non-mounters (N 17)	4	3	NS	2	6	NS	7	6	NS	0	0	NS
B1	Saline 0 4 ml												
	mounters (N 14)	2	4	NS	2	3	NS	6	4	NS	0	0	NS
	non-mounter (N 15)	4	2	NS	3	5	NS	9	5	NS	0	0	NS
B2	Pimozid 100												
	Apomorphine 100												
	mounters (N 14)	4	2	NS	5	6	NS	6	3	NS	0	0	NS
	non-mounters (N 15)	3	6	NS	5	1	NS	6	7	NS	0	0	NS

ing agent phenoxybenzamine did not affect mount or intromission percentage in analogous experiments but increased the number of mounts/min. (FIA-63) Thus neither compounds which increase nor compounds which decrease the noradrenergic tone had any significant influence on the percentage of subjects which displayed pre-ejaculation copulatory behavior provided that the doses used did not too seriously disturb the overt behavior of the subjects However other components of the copulatory behavior (e g the number of mounts/min) seem to be more influenced by the noradrenergic tone

Apomorphine

Apomorphine increased mount percentage and intromission percentage and decreased mount latency The number of mounts/min was increased after 30 μg/kg but decreased after 300 μg/kg (possibly due to the increase in stereotyped motor patterns) Previously it was found that L-DOPA (given after pargyline and MK486 pretreatment) increased mount and intromission percentages and decreased mount latency Thus L-DOPA and apomorphine facilitated components of the copulatory behavior in spite of the fact that both compounds decreased motor activity as measured by the Animex[R] activity meter The facilitatory effects of apomorphine on copulatory behavior were blocked by a dose of pimozide which by itself had no significant effect on copulatory behavior This dose of pimozide also blocked the facilitatory effects of L-DOPA on mount and intromission percentages (to be published) A higher dose of pimozide as well as treatment with the tyrosine hydroxylase inhibitor α-methyl-p-tyrosine decreased mount and intromission percentages increased mount latency and decreased the number of mounts/min. These inhibitory actions were not associated with a decrease in motor activity or other disturbances of the overt behavior Notably pimozide in a dose which inhibited copulatory behavior decreased the scores for female-oriented activity among non-mounters

In contrast these scores were increased after apomorphine 30 and 100 µg/kg These findings suggest that an increased dopaminergic tone facilitates and a decreased dopaminergic tone inhibits both copulatory and non-copulatory components of the male rat s interaction with the female Current data indicate the opposite relationship for drugs affecting the serotoninergic tone

Some studies on the effect of LSD and apomorphine on copulatory behavior in the intact male rat have previously been made Facilitatory effects of LSD on certain components of the established copulatory behavior (Gillett 1960; Soulairac 1963; Bignami 1966) as well as inhibitory ones (Bignami 1966; Uyeno 1967) have been reported. Both facilitatory and inhibitory effects of a fairly high dose of apomorphine (0 8 mg/kg) have been demonstrated by Butcher et al (1969) Since vigorously copulating intact subjects were used in these studies and other parameters recorded meaningful comparisons with the present results are difficult to make

Taken together the data of the present investigation show that LSD decreased the percentage of subjects which displayed pre-ejaculation copulatory behavior In contrast this percentage was increased by apomorphine while clonidine had no effect on this behavioral component When these data are compared with the ones obtained with other neuropharmacological agents (see above) it is suggested that the effects of LSD and apomorphine (on the percentage of subjects which displayed pre-ejaculation copulatory behavior) were due to functionally increased central nervous serotonin and dopamine receptor activity respectively

For generous supplies of hormones and drugs I thank Organon the Netherlands through Erco Sweden (testosterone propionate estradiol benzoate progesterone) Sandoz Switzerland (D-lysergic acid diethylamide, apomorphine HCl) Boehringer Ingelheim Western Germany (clonidine HCl) and Janssen Belgium (pimozide) The work was supported by NIH grant R01 HD04108-03 and the Swedish Medical Research Council grant 14X-64-08 to Dr Bengt Meyerson and grants from the Medical Faculty University of Uppsala to the author

REFERENCES

AGHAJANIAN G K LSD and CNS transmission Ann Rev Pharmacol 1972a 12 157-168

AGHAJANIAN G K Chemical-feedback regulation of serotonin-containing neurons in the brain N Y Acad Sci 1972b 193 86-94

AGHAJANIAN G K W E FOOTE and M H SHEARD Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe Science 1968 161 706-708

AGHAJANIAN G K W E FOOTE and M H SHEARD Action of psychotogenic drugs on single midbrain raphe neurons J Pharmacol Exp Ther 1970 171 178-187

AGHAJANIAN G K H J HAIGLER and F E BLOOM Lysergic acid diethylamid and serotonin: direct actions on serotonin-containing neurons in rat brain Life Sci 1972 11 615-622

ANDÉN N E S G BUTCHER H CORRODI K FUXE and U UNGERSTEDT Receptor activity and turnover of dopamine and n radrenaline after neuroleptics European J Pharmacol 1970a 11 303-314

ANDÉN N E H CORRODI K FUXE and T HÖKFELT Evidence for a central 5-hydroxytryptamine receptor stimulation by lysergic acid diethylamide Br J Pharmac 1968 34 1-7

ANDÉN N E H CORRODI K FUXE B HÖKFELT T HÖKFELT C RYDIN and T SVENSSON Evidence for a centr l noradrenaline re eptor stimulation by cl nidin Life Sci 1970 9 I 513-523

ANDÉN N E A RUBENSON K FUXE and T HÖKFELT Evidence for dopamine r ceptor stimulation by apomorphine J Pharm Pharmacol 1967 19 627-629

BIGNAMI G Pharmacological influences on mating behavior in the mal rat Psychopharmacologia (Berl) 1966 10 44-58

BROEKKAMP C and J M van ROSSUM Clonidine induced intrahypothalami stimulation of eating in r ts Psychopharmacologia (Berl) 1972 25 162-168

CURTIS D R and R DAVIS Pharmacological tudi s upon neurones of lat ral geniculate of the cat Brit J Pharmacol 1962 18 217-246

DIAZ P S H NGAI and E COSTA F ctor aff ting serotonin turnover Advan. Pharmacol 1968 6B 75-92

ELIASSON M A MICHANEK and B J MEYERSON A diff rential inhibitory action of LSD and amphetamin on copulatory behavior in the female rat Acta Pharmacol et Toxicol 1972 31 Suppl 1 22

ERNST A M Mode f action of apomorphine and dexamphetamine on gnawing compul ion in rats Psychopharmacologia (Berl) 1967 10 316-323

FREEDMAN D X Eff ct of LSD-25 on brain s rotonin J Pharmacol Exp Ther 1961 134 160-161

GILLETT E Eff ts f chlorpromazine and D-lysergic a id diethylamide on ex behavior f mal rats Proc Soc Exptl Biol Med 1960 103 392-394

MALMNÄS C O Testosterone-activated copulatory behavior in the castrated male rat Acta physiol scand 1973a Suppl 395 9-46
MALMNÄS C O Monoamine precursors and copulatory behavior in the male rat Acta physiol scand 1973b Suppl 395 47-68
MALMNÄS C O Copulatory behavior in the male rat after impaired monoaminergic neurotransmission Acta physiol scand 1973c Suppl 395 69-95
MALMNÄS C O and B J MEYERSON p-Chlorophenylalanine and copulatory behaviour in the male rat Nature (Lond) 1971 232 398-400
ROOS B E Decrease in homovanillic acid as evidence for dopamine receptor stimulation by apomorphine in the neostriatum of the rat J Pharm. Pharmacol 1969 21 263-264
ROSECRANS J A R A LOVELL and D.X FREEDMAN Effects of lysergic acid diethylamide on the metabolism of brain 5-hydroxytryptamine Biochem. Pharmacol 1967 16 2011-2021
SCHITT H H SCHMITT and S FENARD Evidence for an α-sympathomimetic component in the effects of catapres n on vasomotor centres: antagonism by piperoxane European J Pharmacol 1971 14 98-100
SCHUBERT J H NYBÄCK and G SEDVALL Accumulation and disappearance of ^{3}H-5-hydroxytryptamine formed from ^{3}H tryptophan in mouse brain: effect of LSD 25 European J Pharmacol 1970 10 215-224
SIEGEL S Nonparametric statistics for the behavioral sciences (McGraw-Hill New York 1956)
SOULAIRAC M L Étude expérimentale des régulations hormono-nerveus s du comportement sexuel du Rat mâle Ann d Endocr 1963 24 Suppl to N 3 1-98
UNGERSTEDT U Postsynaptic supersensitivity after 6 hydroxydopmine induced degeneration of the nigro-striatal dopamine system in the rat brain Acta physiol scand 1971 Suppl 367 69-93
UNGERSTEDT U L L BUTCHER S G BUTCHER N E ANDÉN and K FUXE Direct chemical stimulation of dopaminergic mechanisms in the neostriatum of the rat Brain Res 1969 14 461-471
UYENO E T Lysergic acid diethylamide and sexual dominance behavior of the male rat Int. J Neuropsychiat. 1967 3 188-190

MONOAMINERGIC INFLUENCE ON TESTOSTERONE-ACTIVATED COPULATORY BEHAVIOR IN THE CASTRATED MALE RAT

GENERAL SUMMARY AND CONCLUSIONS

This summary is based on the following communications: Malmnäs 1973a (I) 1973b (II) 1973c (III) and 1973d (IV) The four papers will be referred to by the Roman numerals

THE METHOD (I)

Copulatory behavior in the male rat consists of a series of mounts with or without penile intromission into the female vagina terminated by ejaculation This behavior is dependent on gonadal hormones: it disappears gradually after castration and can be restored to the pre-castration level by testosterone propionate (TP) (Shapiro 1937; Stone 1939; Beach and Holz-Tucker 1949) Most likely testosterone activates copulatory behavior in the male rat by a direct action on the central nervous system (Davidson 1966; Lisk 1967)

The main aim of the experiments presented in paper (I) was to develop a procedure appropriate for pharmacological studies on testosterone-activated heterosexual pre-ejaculation copulatory (mounting) behavior in the castrated male rat. One major objective was to find a schedule of TP treatment that induced and could maintain a submaximal response with respect to the percentage of subjects that displayed mounting (mount percentage in this summary also called copulatory response)

A testing procedure was used in which the male was given 5 min of adaptation to the observation cage before a sexually receptive female was introduced The time allowed from the introduction of the female to the first mount was restricted to 3 min If the male mounted the female within 3 min the test continued for another 3 min counted from the first mount or until ejaculation occurred whichever came first Although the method was primarily designed to measure mount percentage other components of the copulatory behavior were also recorded such as the percentages of subjects which achieved intromission and ejaculation the latencies to the first mount and first intromission the average number of mounts/min and the percentage of mounts with intromission of the total number of mounts (intromission ratio) Tests were carried out regularly once weekly

Within 5 weeks from castration the mount percentage had decreased from 100 to 50% In non-mounting subjects mounting behavior appeared 30-54 hours after a single injection of TP irrespective of the dose given (0 10 - 0 80 mg/kg) The duration of the effect of TP on mount percentage was dose-dependent Counted from the day of injection the duration was 5-10 days after a dose of TP that induced a submaximal response (0 10 - 0 20 mg/kg) while after a supramaximal dose of TP (0 40 mg/kg) the duration was longer

A schedule of weekly TP injections of 0 20 mg/kg or less was found to induce and maintain a submaximal response with respect to mount percentage The response was dose-dependent and remained on a stable submaximal level for several months Even after several months of weekly TP treatment the response was sensitive to a change in the amount of TP given

The TP-activated copulatory response was not changed by adrenalectomy However ACTH increased the copulatory response This effect was likely caused by increased adrenal steroid hormone production as no effect of ACTH was seen in adrenalectomized subjects These data demonstrate that although the adrenals did not influence the TP activated response under ordinary conditions it was

possible to increase the response by stimulating the adrenals

A procedure to score the specific orientation of activity was developed as a tool to differentiate specific from non-specific effects of drugs on male copulatory behavior It was found that irrespective of subsequent mounting or non-mounting all males approached and explored the female when she was introduced into the observation cage A negligible amount of time during copulatory tests was devoted to immobility

Previously neuropharmacological studies on heterosexual copulatory behavior in the male rat have exclusively been conducted in intact as a rule vigorously copulating subjects Compared to previous procedures the present method offers the advantage of being independent of drug-effects on the gonadal hormone production Furthermore, the copulatory response can be maintained at a submaximal level which permits a drug-induced increase or decrease of the response to be detected.

NEUROPHARMACOLOGICAL EXPERIMENTS

Increase of the Monoaminergic "Tone (II)

A general increase of the monoaminergic tone achieved by the monoamine oxidase inhibitors pargyline (40 mg/kg) and nialamide (250 mg/kg) induced a decrease of mount percentage A similar decrease of mount percentage was obtained by the serotonin precursor DL-5-HTP (1 0 and 2 5 mg/kg) given after pretreatment with a subeffective dose of pargyline (20 mg/kg) In contrast analogous experiments with the catecholamine precursor L-DOPA (1 0 and 10 mg/kg) did not decrease the copulatory response although motor activity (as measured by the Animex^{R} activity meter) was more decreased by L-DOPA 10 mg/kg than by DL-5-HTP 2 5 mg/kg The difference in effect between L-DOPA and 5-HTP indicates that the pargyline-induced inhibition of the copulatory response was due to increased serotonin levels rather than to an increase in catechol-

amines This is further supported by the fact that pretreatment with the serotonin synthesis inhibitor p-chlorophenylalanine prevented the inhibitory effect of pargyline on copulatory behavior in methodologically identical experiments (Malmnäs and Meyerson 1971) In order to investigate the significance of increased central nervous versus peripheral monoamine levels for the copulatory behavior precursor experiments were also performed with the decarboxylase inhibitor MK486 MK486 does not pass the blood-brain barrier to any significant extent and therefore prevents the peripheral decarboxylation but not the central nervous utilization of the precursors (Porter et al 1962; Bartholini and Pletscher 1969; Henning and Rubenson 1971) The experimental design was analogous to the pargyline + precursor experiments but MK486 was given as well After addition of MK486 to the pargyline pretreatment the difference in effect between DL-5-HTP (2 5 mg/kg) and L-DOPA (2 5 mg/kg) became still more pronounced; whereas mount percentage was decreased after DL-5-HTP the response was increased after L-DOPA. The specificity of this difference in effect with respect to the copulatory response is demonstrated by the effects of the precursors on the overt behavior; the gross appearance and motor activity were unaffected by the dose of DL-5-HTP used while L-DOPA (in this low dose) decreased motor activity and even induced periods of immobility among the subjects In contrast to the increased copulatory response obtained by L-DOPA 2 5 mg/kg the selective noradrenaline precursor DL-DOPS 3 0 10 and 30 mg/kg did not facilitate the response

Decrease of the Monoaminergic Tone (III)

A general decrease of the monoaminergic tone induced by the monoamine depletors reserpine 0 5 mg/kg and tetrabenazine 1 0 and 3 0 mg/kg decreased mount percentage This decrease in mount percentage was not associated with a corresponding decrease in noncopulatory female-oriented activity Like reserpine and tetrabena-

zine the catecholamine synthesis inhibitor α-methyl-p-tyrosine (Spector et al 1965) 75 and 150 mg/kg, decreased the copulatory response α-Methyl-p-tyrosine 75 mg/kg did not decrease motor activity or otherwise disturb the overt behavior In contrast to the inhibitory effects obtained by α-methyl-p-tyrosine the serotonin synthesis inhibitor p-chlorophenylalanine (Koe and Weissman 1966; Jequier et al 1967) given 100 mg/kg for 4 consecutive days facilitated the copulatory response The specificity of this effect is indicated by the fact that p-chlorophenylalanine in this dosage induced a pronounced decrease in motor activity p-Chlorophenylalanine increased mount percentage also in adrenalectomized subjects Thus this effect was not secondary to an increased adrenal steroid hormone production

The effects of α-methyl-p-tyrosine and p-chlorophenylalanine suggest that a decreased catecholaminergic tone inhibits and a decreased serotoninergic tone facilitates the copulatory response

α-Methyl-p-tyrosine inhibits tyrosine hydroxylase which converts tyrosine to DOPA. DOPA is then decarboxylated to dopamine Within certain central nervous pathways dopamine is the main neurotransmittor while in other pathways noradrenaline is the main catecholaminergic transmittor (cf Ungerstedt 1971) Within noradrenergic neurons dopamine is converted to noradrenaline by means of dopamine-β-hydroxylase which in turn is inhibited by the compound FLA-63 (Florvall and Corrodi 1970) This drug provides a means to selectively influence the noradrenaline synthesis

FLA-63 10 mg/kg decreased motor activity but did not decrease mount percentage The inhibitory effect of α-methyl-p-tyrosine on the copulatory response but lack of effect of FLA-63 suggests that an adequate dopaminergic tone rather than noradrenergic tone is essential for the copulatory response This was further demonstrated by experiments with agents known to block catecholaminergic receptors Pimozide which in low doses blocks dopamine but not noradrenaline receptors (Andén et al 1970) inhibited the copulatory response at a dose level (0 25 mg/kg) which did not decrease motor activity or otherwise disturb overt behavior In con-

trast the noradrenaline antagonist phenoxybenzamine (Andén et al 1966 1967) did not decrease mount percentage at a dose which decreased motor activity but otherwise had little disturbing effect on overt behavior (3 0 mg/kg) Chlorpromazine which blocks both noradrenaline and dopamine receptors (Andén et al 1970) induced a decrease in mount percentage both after a dose that increased motor activity (1 0 mg/kg) and after a dose that decreased motor activity (2 5 mg/kg) The adrenergic β-receptor blocking agent propranolol 1 0 and 4 0 mg/kg did not affect mount percentage

The Effect of Monoaminergic Agonists (IV)

In paper IV the effects of LSD clonidine and apomorphine on copulatory behavior were studied The rationale for selecting these agents was the accumulating evidence suggesting that these compounds affect monoamine receptors in a manner which would be expected by receptor stimulating agents (agonists): LSD on serotonin receptors (Andén et al 1968; Aghajanian et al 1968 1970 1972; Eliasson et al 1972; for review see Aghajanian 1972 a b) clonidine on noradrenaline receptors (Andén et al 1970a; Schmitt et al 1971; Broekkamp and van Rossum 1972) and apomorphine on dopamine receptors (Ernst 1967; Andén et al 1967; Ungerstedt et al 1969; Ungerstedt 1971a)

LSD 30 and 100 µg/kg decreased mount percentage At 30 µg/kg the overt behavior was virtually unaffected and there was no effect on motor activity Clonidine 3 10 and 30 µg/kg did not affect mount percentage Apomorphine 30 100 and 300 µg/kg increased mount percentage Both clonidine 30 µg/kg and apomorphine 100 µg/kg decreased motor activity

CONCLUSIONS

The method developed in paper I turned out to be a useful proce-

dure for neuropharmacological studies of pre-ejaculation copulatory behavior

Mount percentage

The current data (from papers II - IV) on the influence on mount percentage of drugs affecting serotoninergic noradrenergic and dopaminergic neurotransmission are summarized in table I In this connection it must be emphasized that these changes in mount percentage have a significance only as long as the treatment regimen does not seriously impair the well-being and/or motor capabilities of the subjects The range of doses used in this investigation fulfilled these requirements: In instances where the copulatory response was decreased this was not associated with a disturbance of overt behavior

When the data of the present investigation are taken together it can be concluded that drugs which increase the serotoninergic tone inhibit the copulatory response while drugs decreasing the serotoninergic tone have the opposite effect. In contrast a drug-induced increase of the dopaminergic tone induces an increased copulatory response while a decrease of the dopaminergic tone inhibits the response There is no support for a similar relationship for drugs affecting the noradrenergic tone

Other copulatory parameters

Among the other copulatory parameters recorded intromission percentage followed essentially the same pattern of monoamine dependence as mount percentage (Notable exception: propranolol decreased intromission percentage but not mount percentage) In general an increase in mount percentage was accompanied by a decrease in mount latency and a decrease in mount percentage by an increase in mount latency On the other hand a change in mount

latency was not necessarily associated with a change in mount percentage: clonidine decreased mount latency and DL-DOPS increased mount latency but neither drug affected mount percentage The number of mounts/min as a rule changed in the same directions as mount percentage however the number of mounts/min was increased by FLA-63 and decreased by clonidine and DL-DOPS The only significant changes in intromission latency was a decrease after L-DOPA and an increase after DL-5-HTP when the precursors were given after combined pargyline and MK486 pretreatment Intromission ratio was increased after L-DOPA both with and without MK486 addition to the pargyline pretreatment and decreased by propranolol Since the times of testing were kept very short only a very small number of subjects normally achieved ejaculation Ejaculation percentage was however significantly increased by p-chlorophenylalanine and by L-DOPA given after combined pargyline and MK486 pretreatment

Orientation of activity

Concerning the influence of the drugs tested on the orientation of activity during copulatory tests it is of particular interest that the female-oriented activity among non-mounting subjects was increased by apomorphine and decreased by pimozide DL-5-HTP (after pargyline + MK486) and LSD These findings suggest that also other components of the male rat s interaction with the female than copulatory may be under a dopaminergic/serotoninergic control in a way similar to the copulatory response

Copulatory behavior in the male and female

Copulatory behavior in the ovariectomized female rat (lordosis) is activated by estrogen followed by progesterone There is abundant evidence that the lordotic behavior displayed by the female

TABLE I Schem ti represe ion of the influe f serotoni no ad enali and dopamine on mount pe centage i cast ted mal ts ai tained on ubno mal response level ith respect to thi pa amet by means f weekly testo t propionate t eatment

AMINE	"TONE" AT RECEPTOR SITES	MOUNT PERCENTAGE	DRUGS TESTED
SEROTONIN	increased	decreased	pargyline 5-HTP pargyline MK486 5-HTP LSD
	decreased	increased	p-chlorophenylalanine
NORADRENALINE	increased	unaffected (?)	pargyline MK486 DL-DOPS clonidine
	decreased	unaffected (?)	FLA-63 phenoxybenzamine
DOPAMINE	increased	increased	pargyline MK486 L-DOPA apomorphine
	decreased	decreased	α-methyl-p-tyrosine chlorpromazine pimozide

when mounted by a male is inhibited by increased serotoninergic tone (Meyerson 1964; for review see Meyerson et al 1973) It thus appears that serotonin has an antagonistic effect on certain components of copulatory behavior in both sexes It is not yet clear however whether also catecholamines are involved in the central nervous regulation of copulatory behavior in the female and if so whether their effect is stimulatory or inhibitory

ACKNOWLEDGEMENTS

This study has been carried out at the Department of Medical Pharmacology University of Uppsala Sweden.

I wish to express my deep gratitude to:

My teacher Docent Bengt Meyerson for guiding me into the fields of neuropharmacology and behavioral methodology and among thousands of things his inspiring enthusiasm,

Professor Ernst Bárány for stimulating leadership of the department and constructive criticism of the manuscript

Mrs Asta Palis Miss Ann-Kristin Söderlund and Miss Marita Berg for skilful technical assistance and Mr Arne Broström for his care of the animals

Mrs Ann-Mari Kjellberg Mrs Mona Nilsson and Miss Monica Nilsson for solidary fighting with the preparation of the manuscript and Mr Manne Fredriksson for photographic details

Members and visitors of the psycho-endocrine group for bright ideas and high spirits; the English was kindly revised by FK Mona Eliasson and Dr Larry Fechter

The sauna group for its existence despite the current trends in society

My wife Ulla for brilliant and ineffable effaquillacy

The work was supported by NIH grant R01-HD4108-03 and the Swedish Medical Research Council grant 14X-64-08 to Dr Meyerson and grants from the Medical Faculty University of Uppsala to the author

REFERENCES

AGHAJANIAN G K, W E FOOTE and M H SHEARD Lysergic acid diethylamide: sensitive neuronal units in the midbrain raphe Science 1968 161 706-708

AGHAJANIAN G K, W E FOOTE and M H SHEARD Action of psychotogenic drugs on single midbrain raphe neurons J Pharmacol Exp Ther 1970 171 178-187

AGHAJANIAN G K H J HAIGLER and F E BLOOM Lysergic acid diethylamide and serotonin: direct actions on serotonin-containing neurons in rat brain Life Sci 1972 11 615-622

AGHAJANIAN G K LSD and CNS transmission Ann Rev Pharmacol 1972a 12 157-168

AGHAJANIAN G K Chemic l-feedback regulation of serotonin-containing neurons in the brain N Y Acad Sci 1972b 193 86-94

ANDEN N E M G M JUKES and A LUNDBERG The effect of DOPA on the spinal cord 2 A pharmacological analysis Acta physiol scand. 1966 67 387-397

ANDEN N E H CORRODI K FUXE and T HÖKFELT Increased impulse flow in bulbospinal noradrenaline neurons produced by catecholamine receptor blocking agents European J Pharmacol 1967 2 59-64

ANDEN N E A RUBENSON K FUXE and T HÖKFELT Evidence for dopamine aceptor timulation by apomorphine J Pharm. Pharmacol 1967 19 627-629

ANDEN N E H CORRODI K FUXE and T HÖKFELT, Evidence f r a ntral 5-hydroxytryptamine recept r stimul tion by lys rgic acid diethylamid Brit J Pharmacol 1968 34 1-7

ANDEN N E S G BUTCHER H CORRODI K FUXE and U UNGERSTEDT Receptor ctivity and turnover of dopamine and nor d enaline af t r neuroleptics European J Pharmacol 1970 11 303-314

BARTHOLINI B and A PLETSCHER Effect of various decarboxylase inhibit on the bral metabolism of dihydroxyphenylalanine J Pharm. Pharmacol. 1969 21 323-324

BEACH F A and A M HOLZ TUCKER Effe ts f diff r nt concentrations of androgen up n sexual b havior in castr ted male rat J Comp Physiol Psychol 1949 42 433-453

BROEKKAMP C and J M van ROSSUM Clonidine induced intrahypothalamic timulation of eating in rats Psychopharmacologia (Berl) 1972 25 162-168

DAVIDSON J M. Activation of the male rat s sexual behavi r by intracerebral implantation of androgen Endocrinol 1966 79 783-794

ELIASSON M A MICHANEK and B J MEYERSON A diff rential inhibit ry tion of LSD and amphetamine on copulatory behavior in the femal t Acta Pharmacol et Toxicol 1972 31 Suppl 1 22

ERNST A M. Mode of a tion of apomorphine and dexamphetamine on gnawing compulsion in rat Psychopharmacologia (Berl) 1967 10 316-323

FLORVALL L and H CORRODI Dopamine β-hydroxylase inhibitors Acta Pharm. Suecia 1970 7 7-22
HENNING M and A RUBENSON A Effects of 5-hydroxytryptophane on arterial blood pressure body temperature and tissue monoamines in the rat Acta pharmacol (Kbh) 1971 29 145-154
JEQUIER E W LOVENBERG and A SJOERDSMA Tryptophan hydroxylase inhibition: the mechanism by which p-chlorophenylalanine depletes rat brain serotonin Mol Pharmacol 1967 3 274-278
KOE B K and A WEISSMAN p-Chlorophenylalanine a specific depletor of brain serotonin J Pharmacol Exp Ther 1966 154 499-516
LISK R.D Neural localization for androgen activation of copulatory behavior in the male rat Endocrinol 1967 80 754-761
MALMNÄS C O and B J MEYERSON p-Chlorophenylalanine and copulatory behaviour in the male rat Nature (Lond) 1971 232 398-400
MALMNÄS C O Testosterone-activated copulatory behavior in the castrated male rat Acta physiol scand 1973a. Suppl 395 9-46
MALMNÄS C O Monoamine precursors and copulatory behavior in the male rat Acta physiol scand 1973b Suppl 395 47-68
MALMNÄS C O Copulatory behavior in the male rat after impaired monoaminergic n urotransmission Acta physiol scand 1973c Suppl 395 69-95
MALMNÄS C O Effects of LSD-25 clonidine and apomorphine on copulatory behavior in the male rat Acta physiol scand 1973d. Suppl 395 96-116
MEYERSON B J Central nervous monoamines and hormone induced estrus behavior in the spayed rat Acta physiol scand 1964 63 Suppl 241 1-32
MEYERSON B J M ELIASSON L H LINDSTRÖM A MICHANEK and A -K SÖDERLUND Monoamines and female sexual behaviour Neuropsychopharmacology 1973 Proceedings of the VIII International Congress of the CINP Copenhagen 1972 352-358
PORTER C C L S WATSON D C TITUS J A TOTARO and S S BYER Inhibition of dopa-decarboxylase by the hydrazine analog of α-methyldopa Biochem. Pharmacol 1962 11 1067-1077
SCHITT H H SCHMITT and S FENARD Evidence for an α-sympathomimetic component in the effects of catapresan on vasomotor centres: antagonism by piperoxane European J Pharmacol 1971 14 98-100
SHAPIRO H A Effect of testosterone propionate upon mating Nature (Lond) 1937 139 588-589
STONE C P Copulatory activity in adult male rats following castration and injections of testosteron propionate Endocrinol 1939 24 165-174
UNGERSTEDT U L L BUTCHER, S C BUTCHER N E ANDEN and K. FUXE Direct chemical stimulation of dopaminergic mechanisms in the neostriatum f the rat Brain Res 1969 14 461-471
UNGERSTEDT U Stereotaxic mapping of the mon amine pathways in the rat brain Acta physiol scand 1971 Suppl 367 1-48
UNGERSTEDT Postsynaptic supersensitivity after 6 hydroxydopamine induced degene ation of the nigro-striatal dopamine system in the rat brain Acta physiol scand 1971a Suppl 367 69-93

Acta Physiologica Scandinavica
Supplementum 396

XIV
Scandinavian Congress of Physiology and Pharmacology
Bergen 1973

Abstracts of symposia, invited lectures and free

Bergen 1973

Acta Physiologica Scandinavica
Supplementum 396

XIV
Scandinavian Congress of Physiology and Pharmacology Bergen 1973

Abstracts of symposia, invited lectures and free communications

Bergen 1973

SUBJECT INDEX

The figures refer to the abstract numbers.

SYMPOSIA

Cyclic AMP 1– 6
Current problems in capillary physiology 7–13
Prostaglandins 14–23
Pharmacological effects of plasma protein binding 24–29
Salt and water homeostasis 30–34
Plasticity in the central nervous system 25–38

INVITED LECTURES

Adaptations to environment of cardiovascular functions 39
Functional properties of cells in the parietal somatosensory and association cortices of the monkey 40
From physiology to pathophysiology — primary hypertension, a disorder of regulation 41
The role of the muscle spindles in voluntary motor activity 42
Regulation of cerebrospinal fluid pH 43
Cholinergic receptors in mammalian skeletal muscle 44
Transport pathways in epithelia 45
The structural basis of contraction in vertebrate smooth muscles 46
Central and peripheral monoaminergic mechanisms 47
Vascular reactions and mobilization of fat in adipose tissue 48
Circulatory factors in intestinal absorption 49
Osmolar control of the circulation 50

FREE COMMUNICATIONS

Sensory physiology 51– 58
Central transmitters I Histochemistry 59– 64
—»— —»— II Biochemistry 65– 73
Transcapillary transport 74– 82
Gastrointestinal functions 83– 91
Spinal cord and cerebellum 92– 97

Exercise physiology	98–109
Central transmitters III Basal ganglia	110–116
Behavior	117–122
Central transmitters IV Behavior	123–134
Kidney I	135–142
Circulation I	143–149
—»— II	150–160
Ion and water transport	161–166
Prostaglandins	167–173
Brain mechanisms	174–180
Endrocrinology	181–189
Myocardium	190–197
Mast cells and histamine	198–203
Kidney II	204–211
Drug mechanisms	212–220
Clinical pharmacology	221–227
Pharmacokinetics	228–238

Lundholm, L (Department of Pharmacology, School of Medicine Linköping, Sweden): CYCLIC AMP AS A SECOND MESSENGER 1

Cyclic AMP (CA) is synthetized (ATP ⟶ CA+PP1 by the enzyme adenyl cyclase and hydrolyzed (CA ⟶ 5 AMP) by phosphodiesterase These enzymes are present in most tissues both in animals and plants Their activity is influenced by hormones and drugs The tissue level of CA is regulated by the combined action of these two enzymes The adenyl cyclase activity is influenced by receptors associated with the enzyme Adrenergic α- and β-effects the action of ACTH glucagon acetylcholine and prostaglandins are mediated by specific receptors, which increase or reduce the adenyl cyclase activity in the tissues Hormones as cholecystokinin prostaglandins and drugs as theophylline, papaverine nitroglycerin and Ca^{++}-ions influence the CA-level through an action on the phosphodiesterase activity A great number of metabolic reactions and physiological processes are influenced by CA They include activation and inactivation of enzymes release of hormones and neurotransmitters influence on secretory processes effects on the contraction-relaxation cycle in heart and smooth muscles and platelet function among others CA activates a kinase which phosphorylates proteins in the presence of ATP and Mg^{++} The nucleotide is bound to a specific CA-binding protein which in absence of CA inhibits the activity of the protein kinase This action has been suggested to be the primary effect of CA in its regulatory function on metabolic processes Sensitive and specific analytic methods for determination of CA have been developed by using the CA-binding protein

Osnes J B , Christoffersen T Øye I (Institute of Pharmacology University of Oslo Norway) MECHANISM OF THE INOTROPIC EFFECT OF CATECHOLAMINES AS REVEALED FROM EXPERIMENTS ON THE PERFUSED RAT HEART

It is now widely accepted that the contraction/relaxation cycle in cardiac muscle is governed by Ca^{++} ions which rhythmically become available for (and are removed from) the acto-myosin-troponin complex The amount of available Ca^{++} is supposed to limit tension development Ca^{++} bound to the sarcoplasmic reticulum is made available for contraction by the action potential and an increase in this Ca^{++} pool may therefore increase the contractile force Cyclic AMP increases the rate and capacity of Ca^{++} binding of cardiac sarcotubular vesicles This effect depends upon protein phosphorylation by a cAMP dependent protein kinase (Kirchberger M A et al J molec cell Cardiol 1972 4 673) Adrenaline and isoprenaline stimulate cardiac adenylate cyclase and increase the tissue level of cAMP Recently it has been found that the adrenergic beta-receptor of cardiac muscle is closely linked to the catalytic site of adenylate cyclase (Lefkowitz R J Levey G S Life Sci 1972 11/II 821) These findings strongly support the hypothesis that cAMP mediates an inotropic effect of catecholamines

We have measured cAMP levels of perfused rat hearts and the formation of ^{14}C-cAMP from ^{14}C-adenine added to the perfusate - inotropic response to adrenaline and isoprenaline was al- associated with increased cAMP When dopamine or phenyl-ine (above $10^{-6}M$) was used to obtain an inotropic response the increase of cAMP was relatively low Phenylephrine ($10^{-5}M$) in the presence of propranolol ($5x10^{-6}M$) however caused an inotropic response without any increase in cAMP level or cAMP turnover rate This finding supports previous observations of inotropic response to catecholamines without an increase in cAMP under certain conditions (Shanfeld J et al J Pharmacol exp Ther 1969 169 315 Øye I Langslet A Adv cycl Nucleo Res 1972 1 281) We cannot exclude the possibility of a local increase in cAMP undetectable by the methods used or of merely a displacement of preformed cAMP A more plausible explanation however, is that the inotropic response to catecholamines also contains a cAMP-independent component which is mediated by adrenergic alfa-receptors

Isoprenaline increased the incorporation of ^{32}P (added to the perfusate as orthophosphate) into a TCA precipitate of heart muscle homogenate Investigations of this phosphorylation process might serve to elucidate the difference between the biochemical events involved in the inotropic response after alfa- and beta-adrenoceptor stimulation

Andersson R G G (Department of Pharmacology School of Medicine Linköping, Sweden): CYCLIC AMP A REGULATOR SUBSTANCE IN SMOOTH MUSCLE FUNCTION AND METABOLISM 3

Cyclic AMP has an important role in smooth muscle function and metabolism After stimulation of beta-adrenoceptors by different doses of isoprenaline there was a correlation between the increase in cyclic AMP and the degree of relaxation The relaxation was also correlated to an increase of the phosphorylase a activity and a reduction of ATP content after isoprenaline and after addition of exogenous cyclic AMP The ATP-reduction but not the increase in cyclic AMP content was dependent on the presence of Ca^{++} The relaxing action of papaverine nitroglycerin and theophylline was preceded by an increased cyclic AMP level and combined with an inhibition of the cyclic AMP hydrolyzing enzyme phosphodiesterase (PDE) The inhibiting action of papaverine and nitroglycerin was especially marked in the microsomal fractions which accumulated Ca^{++} The inhibition of the PDE-activity amounted to 80-90 %

Drugs which stimulated the PDE-activity such as imidazole in colon muscle cholecystokinin and prostaglandin E_2 in gallbladder reduced the cyclic AMP level and contracted the smooth muscles

The contracting action of carbacholine and acetylcholine was in rabbit colon preceded by a decreased cyclic AMP content Secondary to the contraction there was an increase of the nucleotide content Carbacholine reduced the cyclic AMP formation by 50 % in the microsomal fraction which released $Ca^{++}$by inhibition of the adenyl cyclase activity

In the Ca^{++}-poor muscle carbacholine produced a sustained reduction of the cyclic AMP level A sustained reduction of the cyclic AMP level after carbacholine was also observed if the muscle was pretreated with polyphloretin phosphate a prostaglandin antagonist

It was also shown that prostaglandin E_1 relaxed rabbit colon and increased the cyclic AMP level, the cyclic AMP increasing action of PGE_1 was in contrast to that of isoprenaline dependent on the presence of Ca^{++} The possibility might be considered that the secondary increase in the cyclic AMP level following contraction was caused by a release of prostaglandins

Alterations in the cyclic AMP level of smooth muscles are suggested to influence the contraction-relaxation cycle An increase in the cyclic AMP level is suggested to relax the muscle whereas a reduction of the nucleotide level was associated with a contraction The relaxation produced by cyclic AMP was a ATP-utilizing process It is suggested that ATP was utilized when Ca^{++} was bound to microsomal fractions of the muscle

4 Nilsson, K. (Department of Pharmacology, School of Medicine, Linköping, Sweden): Ca^{++}-BINDING AND RELEASE IN SMOOTH MUSCLE SARCOPLASMIC RETICULUM IN RELATION TO CYCLIC AMP

Three microsomal fractions were isolated from rabbit colon smooth muscle by differential centrifugation on a sucrose gradient At a Ca^{++} concentration of 1 26 x 10^{-6}M and at 37°C the fractions accumulated Ca^{++} in presence of ATP and Mg^{++} as shown in the table The Ca^{++}-binding was combined with an increased ATP-hydrolysis In absence of ATP or at 4°C the Ca^{++}-binding was much reduced Sodiumazid (5 mM) did not influence the Ca^{++}-binding but it was reduced with 50 % by salyrgan (3 mM) The Ca^{++}-binding was influenced by the cyclic AMP concentration in the fractions (table) The binding was increased by isoprenaline which stimulated the adenyl cyclase activity most in the 35–45 % fractions The actions of isoprenaline was blocked by sotalol Papaverine which reduced the phosphodiesterase activity of the fractions also stimulated the Ca^{++}-uptake The binding was also increased by cyclic AMP itself which stimulated the ATP hydrolysis Carbacholine and acetylcholine released Ca^{++} from the fractions and reduced their cyclic AMP content The actions were most pronounced in the fractions isolated at 35–45 % These actions were inhibited by atropine

The glycerinated rabbit colon muscle was in the presence of the Ca^{++} -binding fractions contracted on addition of acetylcholine It is suggested that drugs by influencing the cyclic AMP formation in the Ca^{++}-binding fractions stimulate the uptake or release of Ca^{++} and thereby relax or contract smooth muscle

TABLE

Microsomal fractions of rabbit colon isolated by differential centrifugation

Per cent sucrose	35	35–45	45–55
[illegible]cumulation /mg prot/10 min	65 5±8 2	33 8±3 0	4 8±2 1
ATP hydrolysis nmol/mg prot/4 min	216±29	31 7±5 4	10 7±3 9
cAMP-formation (pmol/mg prot/10 min)	17 8±1 2	15 2±1 5	20 7±4 3
PDE-activity (pmol cAMP hydrolyzed/ mg prot/h)	15 5±3 1	8 8±2 7	0 6±0 2
Action on Ca^{++}-binding:			
cAMP (10^{-5}M) (10 min)	+20 8±8 2*	+25 5±6 8*	
Isoprenaline ($5x10^{-7}$g/ml) (10 min)	+4 0±1 3*	+31 8±9 2*	
Papaverine ($5x10^{-4}$g/ml) (5 min)		+12 5±3 8*	
Carbacholine (5 3 x 10^{-7}g/ml) (10 min)	-12 9±5 0*	-30 3±2 6**	

5

Christoffersen, T Osnes, J B and Øye, I (Institute of Pharmacology University of Oslo Norway): CYCLIC AMP FORMATION IN RAT LIVER: ALTERATION IN HORMONE RESPONSE WITH AGE AND DURING TREATMENT WITH A CHEMICAL CARCINOGEN

Several cellular functions change during normal development and during malignant transformation Alterations in the cyclic AMP system may be of particular importance in ontogenesis and oncogenesis because of the fundamental regulatory function of cyclic AMP (Robison et al Cyclic AMP Academic Press 1971 Johnson et al Proc nat Acad Sci USA 1971 68 425) We have examined the tissue levels and the formation and breakdown of cyclic AMP in rat liver from fetal life to adult age and in adult liver during treatment with the chemical carcinogen 2-acetylaminofluorene The experiments disclosed a great similarity between the hormone response pattern of immature and preneoplastic liver in contrast to the normal adult tissue

Adenylate cyclase activity was present one week before birth but the enzyme did not respond to adrenalin or glucagon Two days before birth however both adrenalin and glucagon stimulated the adenylate cyclase During the perinatal period the tissue levels of cyclic AMP the adenylate cyclase activity and the production of cyclic AMP in slices increased rapidly reaching maximal values towards the end of the first postnatal week This peak coincided with a maximal phosphodiesterase activity indicating an increased cyclic AMP turnover in neonatal liver With increasing age the tissue levels and the rate of synthesis and breakdown of cyclic AMP declined In addition the response to adrenalin and glucagon developed differently The stimulatory effect of adrenalin on adenylate cyclase activity and cyclic AMP formation in slices gradually disappeared whereas response to glucagon persisted

During treatment of adult rats with 2-acetylaminofluorene the response to adrenalin reappeared This change which made the hormone stimulation pattern resemble that of immature liver occurred within one week before any histological signs of malignancy appeared

6 Ericsson, E (Department of Pharmacology, School of Medicine, Linköping, Sweden): BETA-ADRENOCEPTOR ACTIVITY AND CYCLIC AMP METABOLISM OF RAT AORTA, VARIATIONS WITH AGE

In rat aorta the relaxing action mediated by β-adrenoceptors is reduced or absent in vessels from older animals Since cAMP mediates this relaxation, it was investigated if the cAMP metabolism was altered Tension was measured isometrically in aortic strips from male rats α-adrenoceptors were blocked by phentolamine (1 0 μg/ml) and the preparations were contracted by serotonine (3 3 μg/ml)

Isoprenaline relaxed aortas from rats 1 months of age and increased their cAMP content significantly Aortas from older animals (3 or 6 months) were relaxed very little or not at all and there was no increase of their cAMP content Exogenous cAMP (0 1 mM) relaxed aortas from young rats markedly but the effect was minimal in aortas from older animals Theophylline relaxed aortas from both young (1 m) and old rats (6 m) but the dose-response curve was shifted to the right in older animals Neither basal nor NaF stimulated adenyl cyclase activity was decreased with increasing age The phosphodiesterase activity was reduced in aortas of older rats

The results indicate that the decreased relaxing action of isoprenaline in aortas from older rats probably depends both on a reduced sensitivity of β-adrenoceptors and a decreased sensitivity of the vessels towards the relaxing effect of cAMP

Sørensen S C M Møller & K Møllgård (Institute of Medical Physiology Dept A & Anatomy Department A University of Copenhagen Denmark): ALCIAN BLUE A LOW MOLECULAR WEIGHT TRACER FOR STUDIES OF THE PERMEABILITY OF INTERCELLULAR JUNCTIONS IN THE BRAIN 7

Proteinbound dyes do not pass from blood into interstitial fluid in most areas of the brain This has been explained by the presence of tight junctions between cerebral endothelial cells These junctions appear in the electronmicroscope as a fusion of adjacent cell membranes in specimens fixed in glutaraldehyde and they seem to encompass the endothelial cells in their whole circumference It is however not known if the tight junctions also represent a fusion of adjacent cell membranes in vivo Therefore it is not known whether smaller molecules may pass through these junctions in vivo

In an attempt to evaluate the dimensions of the tight junctions in the brain we sought a smaller tracer molecule than those which previously have been used We have used Alcian blue which has a molecular weight of 1390 It may be seen in the light microscope but it may also be seen in the electron microscope because it forms a complex with OsO_4

The size of the molecule was measured on a spacefilling molecular model In the equatorial plane the greatest distance from the center to the furthest endgroups is 12 Å The largest distance from the equatorial plane to the furthest endgroups is 4 5 Å In order to evaluate whether the molecule behaves as a monomer in the solutions used in our experiments we calculated Stoke s radius from viscosimetric and densitometric measurements on solutions of Alcian blue in mock cerebrospinal fluid and Locke s fluid The calculated Stoke s radius was 8 Å indicating that Alcian blue indeed behaves as a monomer in these solutions

The toxicity of Alcian blue was assessed by measuring O_2-uptake in brain cortex slices from rats incubated with Alcian blue in different concentrations When slices were incubated in a balanced medium containing 0 1% Alcian blue the concentration used for ventriculo-cisternal perfusion the respiratory rate was not affected for up to 2½ hours of incubation Concentrations of Alcian blue above 0 5% caused a decrease in the respiratory rate

Alcian blue does not pass from blood into brain in those areas of the brain which previously has been shown to be impermeable to proteinaceous substances However Alcian blue does penetrate the tight junctions between ependymal cells in those areas of the brain where the ependyma has been shown to be impermeable to proteinaceous substances Thus the tight junctions do allow passage of a substance with a Stoke s radius of 8 Å

8 Møllgård K & S C Sørensen (Anatomy Department A & Institute of Medical Physiology Dept A University of Copenhagen Denmark): CHANGES IN CAPILLARY PERMEABILITY IN THE BRAIN STUDIED WITH ALCIAN BLUE AS A TRACER

Separating the lumen of a capillary from the brain parenchyma are two continous layers the endothelium and the basement membrane Normal brain capillaries are characterized by a low number of pinocytotic vesicles and by no endothelial fenestrations, reducing the passage of high molecular weight hydrophilic substances across the endothelial cells Due to the presence of tight junctions between neighbouring endothelial cells a trans capillary intercellular passage of these same substances is also prevented

Attempting to assess the capillary permeability to smaller molecules we used Alcian blue as a tracer substance in vivo because it is visible in the light microscope as well as the electron microscope Alcian blue has been used widely in histochemistry as a staining and precipitating agent for heteroglycans Basement membranes which contain polyanionic substances will be stained if the intravascular administered tracer passes through the capillary wall and furthermore a reaction product can be seen electron-microscopically in specimens treated with OsO_4

Alcian blue (50–150 mg/kg) was injected intravenously into anesthetized adult rats rabbits and cats By light microscopy Alcian blue was found extravascularly in those areas of the brain where the capillaries previously have been shown to be permeable to substances with high molecular weight (e g choroid plexus median eminence area postrema subfornical organ) - when the animals were breathing 25% CO_2 in O_2 for 10 - tes prior to and during the injection perivascular basement were stained by Alcian blue in extensive areas of the rain This suggests that the high concentration of CO_2 increases the permeability of the endothelium

In the electron microscopic studies Alcian blue-precipitates were found exclusively associated with the luminal part of the capillary wall in normal brain capillaries No precipitates were seen in the pericapillary basement membranes in these areas but in contrast Alcian blue had penetrated the fenestrated endothelial cells in areas where a high permeability is normally found and was found in the basement membranes and along the collagen fibres in the extended perivascular spaces Alcian blue was definitely found in connexion with the endothelial fenestrations Following exposure to CO_2 Alcian blue-precipitates were seen in pericapillary basement membranes in extensive regions of the brain Under these conditions the precipitates were found in the intercellular spaces between endothelial cells suggesting that the increase in permeability is due to an opening of tight junctions

Westergaard E and M W Brightman (Anatomy Department C, University of Copenhagen Denmark and Section on Neurocytology National Institute of Neurological Diseases and Stroke Bethesda Maryland USA): TRANSPORT OF HORSERADISH PEROXIDASE AND FERRITIN ACROSS NORMAL CEREBRAL ARTERIOLES

Vesicular transport of intravenously injected horseradish peroxidase (HRP) occurs in the normal mouse brain across segments of arterioles with an average diameter of 15-30μ These vessels are pial situated primarily in cerebral and cerebellar sulci and parenchymal where they supply the ventral part of the diencephalon and neighboring areas The transporting segments are often located at arteriolar bifurcations but involve unbranched portions of the vessels as well Three to 30 minutes after the injection of 1 to 50 mg of HRP dialyzed against a balanced salt solution the protein reaches the perivascular basement membrane spaces and in some instances the extracellular clefts of the adjacent neuropil As in cerebral capillaries the endothelial cells of the arterioles are linked by tight junctions impervious to HRP Ferritin which has a larger diameter (110 Å) than HRP and would therefore be even less likely to pass through the junctions was also transported across the arterioles within 3 minutes after the injection of 200 to 500 mg

Perfusion through the cerebral ventricles of norepinephrine (100-800 μg) for 3 minutes appeared to enhance the transport of HRP across arteriolar segments This heightened transport was manifested by the increased density of reaction product and its more extensive spread perivascularly Capillaries remained impermeable to HRP in all animals whether or not they were injected with amine

10 Johansson B (Department of Neurology University of Goteborg Sweden): INCREASED CEREBROVASCULAR PERMEABILITY IN ACUTE ARTERIAL HYPERTENSION

Acute experimental hypertension induced by intravenous injection of aramine results in a blood brain barrier damage as demonstrated by spots of extravasation of Evans blue (Johansson et al Acta Neuropath 1970 16 117-124) The lesions are similar to what has been found in hypertensive crisis in experimental renal hypertension (Byrom Lancet 1954 2 201-211) The permeability change seems to be caused by the distension of the cerebral vessels as a result of the sudden increase of the intravascular pressure An abrupt pressure increase is propagated to capillaries and veins resulting in a concomitant rise of the sagittal venous pressure which indicates an abolished autoregulation (Häggendal & Johansson Acta Neurol Scand 1972 48 265-270) A stepwise pressure increase which gives the arterioles time for an autoregulatory constriction between each step does not damage the blood brain barrier even if the total blood pressure is high

An increase of the cerebral blood flow at high blood pressure levels in dogs after clamping of the aorta has been shown by Ekström-Jodal et al(Europ Neurol 1971/72 6 6-10) In the same experimental model a blood brain barrier damage can be demonstrated also after the blood flow and autoregulation have been normalized (Johansson & Linder to be published)

The concentration of amino acids- leucin and methionine- in damaged as compared to intact areas of the brain in hypertensive animals has been studied at different intervals after the acute pressure increase The amino acids have been given by intracarotid (cf Oldendorf Amer J Physiol 1971 221 1629-1639) intravenous injection In the acute stage a passive increase the amino acids is usually found in damaged areas but preliminary results indicate that there might be a decreased transport of amino acids at a later stage

Subliminal damage of the cerebral vessels may increase their sensitivity to pressure After discrete unilateral irradiation of the brain in rabbits - the irradiation itself not being sufficient to damage the blood brain barrier - a moderate blood pressure elevation results in a marked permeability increase in the irradiated hemisphere as compared to the control hemisphere (Blomstrand Johansson & Rosengren to be published)

Eriksson E Ericson L E Lisander B and R.Myrhage (Departments of Anatomy and Physiology University of Gothenburg,Sweden): 11

MICROCIRCULATION IN THE TENUISSIMUS MUSCLE OF THE CAT

When analyzing skeletal muscle circulation in terms of regulation mechanisms controlling flow distribution and some other local events, it has been found necessary to include the findings from the intravital microscope We have chosen to study the vascular bed of the tenuissimus muscle in the cat When examining the changes in blood flow at graded constrictor fibre stimulation and at administration of vasodilator drugs it became evident that the vascular anatomy of this muscle had to be further clarified The registrations in the intravital microscope were therefore combined with the analyses of the same muscle specimens prepared for light and electron microscopy The vascular architecture was also studied on Indian ink injected specimens prepared according to the method of Spalteholz

The tenuissimus muscle of the cat runs from the sacro-coccygeal bones down to the crural fascia.It is a mixed skeletal muscle about two thirds of the muscle fibres being red The average muscle fibre has a diameter of 44µm The vascular bed is composed of vessels smaller than 100µm

Morphological A-V anastomoses were practically never seen.Arterioles smaller than 10µm and venules smaller than 15µm were devoid of smooth muscle cells No precapillary sphincters were seen.An average capillary was 1015µm long having anastomoses to a neighbouring capillary at every 200µm.At the beginning the diameter was 4 7µm in the middle 5 3µm and at the end 5 9µm The number of capillaries/muscle fibre was 0 95 and the calculated capillary surface area was 0 $9m^2/100cm^3$ of muscle tissue

No vasomotion was seen The average red cell velocity in capillaries was 0 5mm/s with a range from 0 up to 1 5mm/s The blood cells especially the leucocytes seemed to influence the flow velocity in a capillary where the flow rate decreased with an increased hematocrit and vice versa.All vessels furnished with smooth muscle cells showed diameter changes on sympathetic stimulation and administration of vasodilator drugs the most marked ones occurring in arterioles of 15-20µm

12 Nicolaysen, G (Institute of Physiology University of Oslo Norway): TRANSVASCULAR FLUID BALANCE AND PROTEIN PERMEABILITY IN THE MICROVESSELS OF THE LUNG

Some recent investigations have added to the understanding of transvascular fluid balance and protein permeability in the microvessels of the lungs It is generally accepted that Starlings equation for transvascular fluid exchange also applies to the lung The observations of Guyton and Lindsey (Circulat Res 1959 7 649) seemed difficult to explain on that basis: They observed no increases in extravascular lung water content upon increases in left atrial pressure up to 25 mm Hg for up to 2 hrs in intact dogs However Staub and coll (e g Staub N C et al Microvasc Res 1972 4 331; Vaughan T R jr et al Clin Res 1972 20 583) have been able to supply data that probably explain the puzzle They chronically cannulated lung lymphatics in sheep In the unanaesthetized animals they found a nearly linear correlation between microvascular pressure and lung lymph flow with lymph flowing also at normal microvascular pressures They also confirmed Guyton and Lindsey s observations on lung water content No increase in extravascular lung water on increases in microvascular pressure up to 25 mm Hg for at least 2 hrs As lymph flow increased upon increases in microvascular pressure the concentration of protein in the lung lymph decreased Thus two mechanisms will tend to keep the lung dry : a) a lymph flow that increases on demand and b) a lymph protein concentration that varies with lymph flow This last thing probably indicates that the interstitial colloid osmotic pressure varies in such a way that an increase in microvascular pressure will be partially offset by a decrease in interstitial colloid osmotic pressure This last-mentioned mechanism has previously been proposed by Lunde and Waaler (J Physiol 1969 205 1) on the basis of experiments on isolated lungs Staub and coll in their experiments also found a rather rapid blood to lymph equilibration of tracer albumin in the sheep with a half time of about 2-2½ hrs Similar observations were done by Boyd et al (J Physiol 1969 201 567) These data point to a fairly high permeability of the microvessels to proteins On the other hand electronmicroscopial studies with protein tracers failed to show detectable transfer of the protein to the interstitium within 30 min after the intravenous injection (Pietra G G et al Science 1969 166 1643; Schneeberger E E and Karnovsky M J J Cell Biol 1971 49 319; Nicolaysen and Staub (in prep) have qualitatively determined the time course of the vascular-extravascular equilibration of albumin in mice lungs using the bright red fluorescence of Evans blue labelled serum albumin Equilibration appeared to be reached within 3 5 hrs of the i v injection of the tracer substance Interstitial tracer was observed already 20 min after the injection Also autoradiographic studies with ^{125}I albumin suggested advanced equilibration at 4 hrs after the injection This study thus lends support to the notion that the permeability of the microvessels in the lungs to proteins is not negligible

Groth T and N A Lassen (Uppsala University Data Center, Uppsala Sweden and Department of Clinical Physiology, Bispebjerg Hospital Copenhagen Denmark): 13

DOES THE GLOMERULAR MEMBRANE OPERATE AS A MILLI-PORE FILTER?

In a previous study of the human glomerular membrane (Arturson G Groth T and Grotte G Clin. Sci 1971 40:137) it was shown that a strict non-linear analysis of dextran sieving data in terms of a two-pore model leads to a surprisingly low value of the mean transglomerular pressure difference (less than 1 mmHg) The corresponding value of the effective pore area was found to largely exceed the total capillary area as estimated by histological techniques (Vimtrup, B J Amer. J. Anat., 1928 41:123)

The calculations yield an estimate of the surface area per unit path length $A/\Delta x$ The discrepancy mentioned was caused by inserting the entire thickness of the glomerular basement membrane (about 2000 Å) in the $A/\Delta x$ expression

In the present communication the discrepancy is discussed If the critically narrow part of the channels of the glomerular filter is of the order 10–50 Å and not 2000 Å then values for the effective pore area can be obtained that are compatible with anatomical estimates. The remaining part of the glomerular basement membrane must then have considerably wider dimensions of its presumably mesh-like system of water filled channels through which the ultrafiltrate passes This solution gives quantitative support to the hypothesis that the glomerular membrane should operate like a millipore filter Such filters have a critically narrow part of its channels occupying only a very small fraction of the thickness of the filter The critically narrow part must face the solution from which ultrafiltrate is formed the filter simply cannot function in the opposite direction because the impermeant solute will clog it up if one tries to reverse the filter

Thus we are led to suggest that also in the glomerulus the true filter that practically excludes colloids from sieving into the ultrafiltrate, is placed on the blood side (in the fenestrae) The remaining part of the filter essentially constitutes a mechanical support through which the ultrafiltrate passes without further being modified In this hypothesis the electronmicroscopical demonstration of some large tracer molecules penetrating fairly deep into the glomerular membrane is not indicative of the typical mode of operation but rather of imperfections in the filter occasionally letting larger molecules penetrate deeper than the critical surface layer

14 Bergström Sune (Karolinska institutet 104 01 Stockholm):
THE PROSTAGLANDINS AND THEIR METABOLISM

During recent years the involvment of the prostaglandins in the regulation in many physiological processes has been studied

Natural prostaglandins and some new analogues show great promise as drugs in several fields of medicine

A short review will be presented on recent developments that might be of interest as a background for the other contributions to the symposium

Änggård E and C Larsson (Department of Pharmacology Karolinska Institutet S-104 01 Stockholm 60 Sweden)
PROSTAGLANDIN MEDIATED HYPOTENSIVE EFFECTS OF ARACHIDONIC ACID IN THE RABBIT

The effect of arachidonic acid (C 20:4) a prostaglandin precursor was studied on the blood pressure and the urinary output of prostaglandins (PG) in rabbits C 20:4 induced a dose dependent decrease in the blood pressure Intraarterial infusion of 15-25ug/kg/min reduced the blood pressure by 10±2% (S D) 30-45ug/kg/min by 21±3% 50-90ug/kg/min by 34±6% and 95-150ug//kg/min by 61±9% The urinary PG identified by bioassay in combination with chromatography and 15-hydroxyprostaglandin dehydrogenase inactivation increased by 70% (n=3) Following indomethacin (10mg/kg) or 5 8 11 14-eicosatetrayonic acid(10mg//kg) two structurally different PG synthesis inhibitors the blood pressure increased by 20±1% ($p < o$ ool) and subsequent infusions of C 20:4 (50-200ug/kg/min) failed to elicit any hypotensive effects or an increase in the urinary PG The results suggest a role for endogenous PG in regulating the blood pressure of the rabbit

Eliasson R H Bygdeman and P Eneroth Dept Physiology Karolinska Institutet and Dept Obstetrics & Gynecology Karolinska Hospital Stockholm Sweden): EFFECTS OF HORMONES ON PROSTAGLANDINS IN HUMAN SEMEN 16

The aim was to investigate effects of exogenous hormones on the content of various prostaglandins (PG) in human seminal plasma Patients referred due to infertility were selected for the study only if there were medical reasons for endocrine treatment A few volunteer medical students were included in some experiments Gonadotropins were given as i m injections of Humegon (75-150 IU HMG 3 or 6 times a week) and/or Pregnyl (500-1500 IU HCG at the same time intervals) for 3 to 6 months Androgens were given either orally (methyltestosterone 10 to 30 mg a day for 10 to 30 days) or i m in the form of longacting preparations (Triolandren/CIBA or Testoviron-Depot/Schering AG) every third week

The seminal plasma was analysed chemically f r PGE PGA + PGB and 19OH-PGA + 19OH-PGB; in most cases also acid phosph tase activity zinc and fructose

In most patients the PG content was not significantly changed by administration of the various hormones In some patients the seminal PG content d creased Nor were the other chemical parameters - as a rule - altered

The results are at variance with thos reported by Sturd (Arzneim -Forsch 1971 21 986) who used bio-assay on isolated guinea-pig ileum and report d that gonadotropin treatment caused a doubling of the PG-activity in human seminal plasma

Factors controlling the occurence of different PG in human semen warrent further studie

Hedqvist, P. (Department of Physiology Karolinska Institute Stock holm Sweden): ASPECTS ON PROSTAGLANDIN MEDIATED CONTROL OF ADRENERGIC TRANSMITTER RELEASE 17

Various mechanisms have been envisaged for the control of trans mitter homeostasis in adrenergic nerves This paper describes the in fluence on noradrenaline (NA) release from adrenergic nerves by pros taglandins (PGs) and agents affecting th α receptors

Isolated guinea pig vasa deferentia preincubated with ^{3}H NA were superfused with Tyrod s solution and were transmurally stimulated (NS) with square wave pulses NS caused th release of NA and of small amounts of PGE lik material V ry low doses of PGE_1 and PGE_2 caused a reversible and dos dependent inhibition of NA overflow in re sponse to NS The inhibition vari d inversely with the stimulation fre quency and with the calcium concentration in the external medium. In hibition of local PG formation increased NA overflow in response to NS α r ceptor blocking agents inc eased and compounds with α receptor stimulating properties decr ased NA overflow in response to NS even after proper pharmacological blockad of NA uptake mechanisms and of local PG formation

It is suggested that NA rel ase induced by nerve action potentials activates p esumed pr synaptic α r c ptors and triggers PG formation and that both the α rec pto and the PG are capable to modulate further r leas of NA from the neuron.

18 Janson, P O and K. Ahrén (Department of Physiology and Department of Obstetrics and Gynecology University of Göteborg Sweden): EFFECTS OF PROSTAGLANDINS ($F_{2\alpha}$) ON OVARIAN BLOOD FLOW

It has been proposed that prostaglandin $F_{2\alpha}$ exerts its luteolytic action by a vascular mechanism, leading to relative ovarian ischemia In the present study anesthetized pseudopregnant rabbits on days 5-9 of luteal phase received $PGF_{2\alpha}$ i.v at a dosage of 50 µg/kg. Before during and for 30 min after PG administration heart rate and systemic arterial pressure were monitored

In one group of animals the blood flow of the ovarian vein was measured during the above mentioned time period. In another group the ovarian blood flow was measured by means of ^{169}Ytterbium and ^{46}Scandium-labelled 35 ± 5 µ microspheres before and at different time periods after PG injection.

$PGF_{2\alpha}$ i.v caused a marked arterial hypotonia and bradycardia for 20-30 min. These effects were then normalized. This response to PG could be modified by atropine i.v or by vagotomy

There were acute reductions in blood flow of the ovarian vein, the whole ovary and the corpus luteum during the phase of arterial hypotonia. The blood flow values returned to their original levels at the same time as the systemic pressure was normalized

In these studies of the rabbit ovarian blood flow no specific effect of i.v $PGF_{2\alpha}$ could be observed.

T C and E Änggård (Department of Pharmacology Karolinska Institutet S 104 01 Stockholm 60 Sweden)
INCREASED JUXTAMEDULLARY BLOOD FLOW FOLLOWING STIMULATION OF INTRARENAL PROSTAGLANDIN BIOSYNTHESIS

The renal blood flow distribution was studied before and during stimulation of intrarenal prostaglandin (PG) formation by infusion of the precursor arachidonic acid (C20:4)

C20:4 was infused into the aorta above the origin of the renal arteries The regional distribution of renal cortical blood flow was determined by injecting ^{51}Cr and ^{141}Ce labelled microspheres (15 ± 5µ) into the left ventricle before and during arachidonate infusion respectively The count rates of ^{141}Ce to ^{51}Cr were determined in consecutive slices from cortex to inner medulla Urine was collected from the urethers and analyzed for prostaglandins using bioassay in combination with specific enzymatic inactivation and silicic acid and gas liquid chromatography

Non-hypotensive doses of C20:4 increased the blood flow in the juxtamedullary cortex by 25 ± 5% and caused an increase of 50 ± 16% of the output of urinary PG mostly PGE_2 Indomethacin decreased the juxtamedullary blood flow by 37 ± 6% and the urinary PG by 42 ± 12% and antagonized the effects of C20:4 on the renal blood flow and urinary PG Our results provide evidence for a role of intrarenal PGE_2 as a mediator of autoregulation of renal blood flow

Søndergaard, J (Department of Dermatology Rigshospital University of Copenhagen Denmark): CUTANEOUS INFLAMMATION INDUCED BY PROSTAGLANDIN E_1 20

The effect of prostaglandin E_1 (PGE_1) on human cutaneous vasculature skin histamine and dermal connective tissue has been studied A single intradermal injection of PGE_1 caused a persistent dusky erythema and a well-defined weal The relationship of the magnitude of the erythema and the weal to dosage of PGE_1 was established in 16 subjects Intradermal injection of 1 µg PGE_1 reduced skin histamine in 10 subjects by approximately 24% Part of the cutaneous reaction to PGE_1 was probably due to the observed endogeneous histamine release since the weal but not the erythema, was reduced by pretreatment with antihistamine Seven injections of 5 µg PGE_1 given intradermally at intervals of 8 hours resulted in a cutaneous reaction clinically characterized by redness edema and tenderness Microscopic examination of the PGE_1-treated skin revealed a moderate mononuclear-cell infiltrate located around the dermal vessels and epidermal appendages Biochemical analyses of PGE_1-treated skin biopsies revealed an increase in the concentration as well as in the total amount of hyaluronic acid as compared with that in saline-treated control biopsies

The results indicate that PGE_1 may initiate regenerative processes in human dermal connective tissue

This work was supported by a grant from the Danish Medical Research Foundation

Strandberg, K (Department of Pharmacology Karolinska Institutet 104 01 Stockholm 60 Sweden : BRONCHOCONSTRICTOR ACTIVITIES OF HISTAMINE PROSTAGLANDIN $F_{2\alpha}$ AND SLOW REACTING SUBSTANCE 21

Bronchoconstricting agents, such as histamine prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and slow reacting substance (SRS) appear in anaphylactic reaction in guinea-pig lung tissue (Piper P J and J R Vane Nature 1969 223 29) In the present investigation the effects of these substances on the airway pressure in anaesthetized guinea-pigs were compared using the Konzett-Rössler technique

Intravenous injection or aerosol administration of the constricting agents caused a dose-dependent increase in airway pressure The onset and duration of action were longer for $PGF_{2\alpha}$ and SRS than for histamine The systemic blood pressure was usually increased by i v injections of $PGF_{2\alpha}$ and SRS whereas when influenced it was lowered on aerosol administration of all the substances The approximate ratio between the dose needed to produce the same peak effect (airway pressure) on i v injection as on aerosol administration was: 1 (0 1-2 5) for histamine 28 (5-100) for $PGF_{2\alpha}$ and 3 (0 3-10) for SRS

It is concluded that histamine $PGF_{2\alpha}$ and SRS can produce an increase in the airway pressure in the guinea-pig and that the magnitude of this effect is dependent on the administration route chosen

22 Svanborg N Hamberg, M. Hedqvist P (Med. Dept. Nacka Hospital and Depts Med. Chem and Physiol Karolinska Institute Stockholm Sweden) ASPECTS ON PROSTAGLANDIN ACTION IN ASTHMA.
Prostaglandins (PGs) are normal constitutents of lung tissue and are released from isolated guinea pig and rat lung by various stimuli This paper describes the effect of inhaled PGFs on airway conductance (SG_A) and of inhaled specific allergen on urinary excretion of PG in patients with allergic asthma

PGs and allergens were administered as aerosols and SG_A was measured in a whole body plethysmograph, 5 α 7α dihydroxy 11 keto tetranor prostane 1 16-dioic acid the major urinary metabolite of $PGF_{1\alpha}$ and $PGF_{2\alpha}$ was determined by multiple ion analysis

$PGF_{2\alpha}$ reduced SG_A in both healthy subjects and asthmatic patients the latter being approximately 10000 times more sensitive to the compound. $PGF_{2\beta}$ (the stereoisomer of $PGF_{2\alpha}$) did not elicit bronchoconstriction but rather a modest increase in SG_A Moderate allergen provoked asthmatic attacks did not significantly alter urinary excretion of the PGF metabolite while an unexpectedly severe and longlasting attack almost doubled the excretion Premedication with indomethacin almost completely abolished the excretion of the PGF metabolite in urine and meanwhile markedly reduced the sensitivity to allergen provocation.

Hyperreactivity to $PGF_{2\alpha}$ possibly coupled with overproduction of the compound is suggested to be of significance in the pathogenesis of allergic asthma

23 Wennmalm Å and M Junstad (Fysiologiska Inst I Karolinska Inst Stockholm Sweden) ENDOGENOUS PROSTAGLANDIN MEDIATED INHIBITION OF PARASYMPATHETIC NEUROTRANSMISSION IN THE RABBIT HEART ?
It has earlier been shown that the release of noradrenaline from sympathetic nerve endings is restricted by an endogenous prostaglandin (PG) mediated control mechanism and that the release of acetylcholine (Ach) from parasympathetic nerves is inhibited by exogenously administered PGE_1 (Wennmalm Å Acta physiol scand 1971 83 Suppl 365) A prerequisite for the endogenous control of sympathetic transmitter release is liberation of PG in response to nerve stimulation In the present paper we wish to report release of PG following parasympathetic nerve stimulation or Ach infusion

Rabbit hearts with intact vagal nerve supply were perfused with Tyrode solution according to the Langendorff technique Right and left vagal nerves were stimulated supramaximally at a frequency of 5 Hz for 10-12 min Perfusate from the heart was collected both at rest and during nerve stimulation In some hearts Ach was infused (28-96 μg during 7-12 min) Lipids in the perfusate were extracted with ethyl acetate They were identified using *T L C* and quantified on a superfused rat stomach At rest a small outflow of PG (4 5 ng/min) from the heart was observed During vagal nerve stimulation this outflow was increased to 11 5 ng/min (39 pg/imp) Infusion of Ach also facilitated the liberation of PG to 13 5 ng/min (2 ng/μg Ach infused) Atropin 1 mg/l neither influenced the nerve stimulation nor the Ach infusion induced outflow of PG Since exogenous PG can inhibit Ach liberation in this organ the present experiments suggest that parasympathetic neurotransmitter release in analogy with sympathetic is restricted by endogenously synthetized prostaglandins

Jacobsen S (Institute of Pharmacology B University of Oslo, Norway): METHODS AND EXPERIMENTAL MODELS IN PROTEIN BINDING STUDIES 24

The interactions of organic compounds with macromolecules are of general interest in biology However the experimental results concerning binding parameters are not always directly relevant to in vivo conditions and results from different laboratories are not compareable due to the different methods used to evaluate binding parameters

The aim of this paper is mainly to give a survey of the different methods used to determine protein binding and to discuss the problems and the pitfalls in the evaluation of binding characteristics as the number of binding sites and the strength of binding The influence of inorganic ions and endogenous substances on the binding will be demonstrated

The binding characteristics of different drugs to serum albumin are presented to elucidate the general topics of the paper

Nilsen O G (Institute of Pharmacology B University of Oslo Norway): THE BINDING OF QUINIDINE TO SUBFRACTIONATED HUMAN SERUM PROTEINS 25

It has been shown previously that many drugs are bound to serum albumin to a large extent However the total binding of many drugs in the human serum are not explained by binding to albumin only Human serum has been fractionated by gel-filtration and ultracentrifugation The binding of quinidine to the proteins was determined by equilibrium dialysis

Our studies demonstrate that albumin is only one of the important drugbinding proteins in the human serum As for the weak base quinidine it seems that of the total quinidine bound in normal human serum 50% is bound to albumin and the other serum fractions of higher molecular weight account for the remaining 50% The serum lipids other than FFA seem to interact with the binding of quinidine to serum proteins

It is concluded that serum albumin the high molecular fractions and the lipids must be considered to evaluate the binding of drugs in serum

26 Frenstad D (Institute of Pharmacology University of Oslo Norway): INFLUENCE OF PLASMA AND TISSUE BINDING ON THE DIS TRIBUTION OF QUINIDINE AND PHENYTOIN IN THE RAT

The plasma protein binding of the basic drug Quinidine and the acidic drug Phenytoin was increased and reduced respectively in rats with acute uremia induced by ligation of the ureteres The binding of the drugs in plasma and tissue homogenates from normal and uremic rats was determined by equilibrium dialysis - The in vivo distribution of the drugs was studied at an apparent equilibrium after i v injections in normal and uremic rats

The distribution of the drugs was expressed as the ratios R_t = total concentration in tissue/total concentration in plasma and R_f = total concentration in tissue/concentration of unbound drug in plasma R_t was different and R_f the same in normal and uremic rats An estimate of the drug distribution to the heart based on the binding parameters obtained in homo genates was in accordance with the distribution in vivo for Phenytoin but not for Quinidine The last discrepancy can be eliminated if one assumes an intracellular pH of 7 3 or cell membranes permeable to both ionized and unionized Quinidine

The findings indicate that the plasma protein and the tissue binding of the drugs determine distribution to the heart The observed changes in tissue distribution in uremic rats are mainly due to changes in plasma proteins

27 Odar-Cederlöf I, and Borgå O (Department of Internal Medicine Karolinska Hospital and Department of Clinical Pharmacology, Huddinge University Hospital Stockholm Sweden): PLASMA PROTEIN BINDING AND PHARMACOKINETICS OF DIPHENYLHYDANTOIN IN UREMIC PATIENTS

Plasma protein binding of diphenylhydantoin (DPH) was lower in uremic patients than in normal persons This seemed to depend mainly on a decreased affinity constant of albumin for DPH in uremia Pharmacokinetic investigation using single intravenous doses showed that in uremic patients whose unbound fraction of DPH was higher total plasma DPH concentrations were lower and values of the apparent volume of distribution V_d higher than in normal subjects Plasma concentrations and values of V_d for unbound drug were similar in the two groups Thus equivalent doses give rise to equivalent unbound concentrations of drug and probably to the same effects but total plasma concentrations differ in the two groups causing a different total plasma concentration - effect ratio in uremic patients

28 Borgå, O (Department of Clinical Pharmacology at Karolinska Institutet, Huddinge University Hospital S-141 86 Huddinge, Sweden): PHARMACOLOGICAL CONSEQUENCES OF PLASMA PROTEIN BINDING

The importance of plasma protein binding of a drug for its pharmacological effects is often overemphasized One reason for this might be that results obtained in vitro have been directly extrapolated to the in vivo situation, without paying attention to the actual pharmacokinetic parameters of the drug

The apparent volume of distribution, V_d of the drug seems to be a parameter which is of great value in predicting the consequences of a change in the plasma protein binding caused by e g a displacement interaction Thus a potentiation of the pharmacological effect is theoretically possible only for drugs with a low V_d (0 10 l/kg) For a drug with a high V_d the displacement interaction will lead to decreased plasma concentrations without significantly increased unbound drug levels in the body At an equilibrium state the decreased plasma protein binding for such a drug might not significantly effect metabolic or renal elimination rates or pharmacological response

29 Dahl, S G (Institute of Pharmacology University of Oslo): RELEVANCE OF PROTEIN BINDING FOR PHARMACOKINETIC MODELS

In clinical pharmacology pharmacokinetic models have proved to be valuable tools for describing the time-course of drug concentrations in blood and urine after administration of single and multiple doses

In a pharmacokinetic model the absorption, distribution and elimination of drugs are described in simple mathematical terms and the reliability of the result depends on how accurately these processes are described It is well known that the binding of drugs to plasma and tissue proteins may influence their pharmacokinetics as for example in the interaction between warfarin and phenylbutazone (O Reilly R A and Levy, G J Pharm Sci 1970 59, 1258)

Some pharmacokinetic models that take into account the binding of drugs to plasma proteins will be presented Unfortunately their usefulness is reduced by complicated mathematical treatment

Practically simpler models which do not account for drug protein binding may have to be used The assumptions involved in these models will be discussed and methods for testing their validity will be given

30 Olsson, K (Dept of Physiology Veterinärhögskolan Stockholm Sweden)

Na^+ IONS AS CENTRAL NERVOUS STIMULI IN SALT AND WATER HOMEOSTASIS

The original and classical evidence for the existence of osmoreceptors in the hypothalamic region of the brain and for a central osmometric control of water balance was obtained by studying the effects of alterations in the solute composition of the blood plasma (Verney E B Proc roy Soc B 1947 135 25) Later studies of central sodium-angiotensin interaction performed with the stimuli applied from the inside of the blood-brain barrier (BBB) suggest that a possible alternative to hypothalamic osmoreceptors may be receptors in the close vicinity of the third cerebral ventricle which respond to changes in the Na^+ concentration of the cerebrospinal fluid (CSF) (Andersson B Amer Scientist 1971.59.408)

Recent studies of osmotic and other stimuli applied both from the outside and from the inside of the BBB in the conscious goat seem to contradict the osmoreceptor theory and support the idea of a periventricular Na^+ sensitive receptor system of importance in the control of water and salt balances A rise in the carotid blood osmolality obtained by infusions of hypertonic NaCl and fructose acts as a much more potent stimulus to thirst and release of antidiuretic hormone (ADH) than the equivalent rise elicited by glucose galactose glycerol and urea A rather effective BBB exists for all these substances (Crone C Acta physiol scand 1965 64 407 ; Yudelevich D L and de Rose N Amer J Physiol 1971 220 841) and their intracarotid application in hypertonic solutions ought to cause approximately the same degree of brain dehydration Therefore the observed differences in dipsogenic and antidiuretic effects appear incompatible with the idea that osmoreceptors are located inside the BBB Further evidence against this idea is the observation that infusions of hypertonic fructose and sucrose into the cerebral ventricular system neither elicit thirst nor release of ADH in contrast to corresponding infusions of hypertonic NaCl

An alternative would be that cerebral osmoreceptors of importance in fluid balance were located outside the BBB or in a region of the brain which lacks an effective barrier of this kind However slow infusions of iso or hypertonic solutions of sucrose or monosaccharides into the lateral cerebral ventricle repress the dipsogenic antidiuretic and natriuretic responses to intracarotid infusions of hypertonic NaCl This would hardly be the case if the intracarotid infusions of hypertonic NaCl acted via receptors located outside the BBB Rather this repressive effect of non-electrolytes infused into the ventricular system seems to be due to a dilution of the CSF Na^+ concentration since no repression of the effects of an intracarotid NaCl infusion is obtained when the non electrolytes are administered into the ventricle dissolved in isotonic saline

Adding small amounts of angiotensin II to intracarotid infusions of hypertonic NaCl and fructose potentiates the ADH releasing and dipsogenic responses If this potentiation reflects a central sodium-angiotensin interaction it indicates that the intracarotid infusions of hypertoni c fructose act dipsogenic and antidiuretic indirectly by inducing a rise in the CSF Na^+ concentration The eventual demonstration that intracarotid infusions of hypertonic fructose (in contrast to equivalent infusions of other monosaccharides) raise the CSF Na^+ concentration would provide additional support for the idea that a periventricular Na^+ sensitive receptor system plays an important role in the central control of body fluid homeostasis Furthermore it would indicate that the blood-liquor barrier is of greater importance for the central control of fluid balance than is the BBB

Bie P (Institute of Medical Physiology C University of Copenhagen Denmark): CONTROL MECHANISMS OF ADH SECRETION

The antidiuretic hormone under normal circumstances is the principal factor in the regulation of renal water excretion This hormone therefore participates in the maintenance of both extracellular volume and strength The stimuli which determine the secretion rate of ADH are still incompletely characterized although the total osmotic activity of the extracellular fluid (TOA) and the (thoracic venous) blood volume frequently are described as the dominating factors corresponding to the dual nature of the effect of ADH

Blood volume Initiated with the findings of Leaf & Mamby (1952) evidence has now accumulated to a degree that makes the significance of this stimulus appear unambiguous The regulatory mechanism depends on stretch receptors located in the left atrium in connection with intact vagus nerves making the fullness of the low pressure system influence the ADH secretion rate The role of high-pressure baroreceptors in the normal regulatory range seems insignificant but these receptors (carotid sinus aorta) have been found to affect ADH release under certain conditions

Osmotic activity: The experiments of Verney (1947) might suggest that TOA influences the release rate of ADH, but the results are only convincing with respect to unphysiologically high stimulus intensities Later work on the subject is scanty and hardly conclusive so the theory of osmoreception is still open to criticism

In order to evaluate further the role of the so-called osmoreceptors a technique has been developed which is based on Verney s principle (intracarotid infusions of hypertonic solutions into hydrated dogs) modified in several ways: a) light chloralose anesthesia was used b) hydration fluid was a hypotonic solution given intravenously c) hydration was maintained (3-4 % BW) by means of a weighing operating table controlling an infusion pump (Jørgensen & Bie (1972)), d) experiments were also performed on dogs in which selective cardiac vagotomy had been made previously in order to avoid volume receptor input on the neurohypophysis The dogs respond properly to the U S P reference standard (80 µU ADH/kg BW bolus injection) and therefore offer an opportunity of repetition and evaluation of the results of Verney

References:
Jørgensen T & Bie P : IEEE trans Bio med Engin 19(1972)246
Leaf A & Mamby A R : J Clin Invest 31(1952)60
Verney E B : Proc Roy Soc Ser B : 135(1947)25

32 Bojesen E (Inst of Experimental Hormone Research University of Copenhagen Denmark): INTRARENAL CONTROLLING SYSTEMS THEORIES AND FACTS

Research into intrarenal control systems specifically involving tubular processes will undoubtedly be intensified when the implications of the load independency of proximal reabsorption and nearly complete filtration equilibrium across the glomerular membrane become fully realised As pointed out almost 20 years ago such phenomena emphasize that the rate of formation and composition of the urine is the resultant of two kinds of properties of the entire nephron i e reabsorptive capacities and fluid flow characteristics both of which are greatly changing along the nephron By simply considering finality or selection one can predict that such a complicated arrangement must be equipped with servo control functions in order to be reliable since in their absence any setting of one of the two kinds of nephron prop rties will greatly influence the result of any change in the other

Most work on intrarenal control mechanisms have until recently been concerned with the juxtaglomerular apparatus (JGA) and the renin system with tubules and/or vessels as targets in a short feedback loop Our observations on interstitial cells (IC) of the medulla suggest however the existence of a second regulatory system Morphologically this extensive cellular system is equipped as secretory cells with lipid droplets as the characteristic constituent The main compon nt of the droplets is triglycerides which in rodents has a high content of polyunsaturated fatty acids a major fraction of which is adrenic acid (22 4 ω 6) This acid is produced in vitro by chain elongation of arachidonic acid (20:4 ω 6) of local origin The numb r of lipid droplets per cell at the tip of the papilla increases rapidly after water or saline loading Thus far no product of known biological activity has been identified although the cells have been assumed to produce prostaglandins More knowledge about the prostaglandins in general and also their relationship to the kidney now makes it unlikely that the cells are merely pro taglandin producing Thus our observation that the adrenic acid is produced and incorporated into triglycerides of the droplet and phosphatides indicates a trapping mechanism for the prostaglandin precursor arachidonic acid

Three results in our recent studies on the sterologenesis of papillary tissue are consistent with the idea that a member of this lipid class is important perhaps locally The rate of sterologenesi is high sterols are not esterified locally and desmosterol is apparently an end product accumulated in the tissue

Unfortunately it is still premature to postulate any particular role of the IC system but we consid r it entirely possible that there is ov rl pping and/or cooperation with th JGA system

Kiil Fredrik (Institute for Experimental Medical Research University 33 of Oslo Ullevål Hospital Oslo Norway) REGULATION OF SODIUM EXCRETION BY EXPANSION OF THE EXTRA-CELLULAR VOLUME.

The rise in sodium excretion after salt intake is associated with expansion of the extra-cellular volume Natriuresis is partly due to a rise in glomerular filtration rate (GFR) and partly to inhibition of proximal tubular reabsorption To distinguish between changes in passive and active energy-requiring tubular transport we have in addition to measurements of renal oxygen consumption used the heat accumulation technique to estimate variations in metabolic rate in cortex and outer medulla Changes in GFR in dogs and corresponding changes in proximal tubular reabsorption (glomerulotubular balance) were associated with only small changes in renal energy requirement (Sejersted et al Am. J Physiol 220: 1488-1493 1971 Lie et al Am J Physiol in press) In contrast a rise in sodium reabsorption (and free water generation) in the ascending limb of Henle s loop during increased delivery from the proximal tubules is associated with corresponding increments in metabolic rate in the outer medulla Thus saline loading increases GFR and inhibits proximal tubular net reabsorption without altering active transport The increased delivery to the distal nephron is partly compensated for by a rise in active reabsorption in the ascending limb of Henle s loop presumably mainly by raising Na-K-ATPase activity This effect is blocked by ethacrynic acid and ouabain

After inhibition of sodium reabsorption with ethacrynic acid the distal nephron is expanded and the hydrodynamic resistance of the nephron reduced towards minimum, under these conditions a change in urine flow during saline and mannitol infusion or ureteral obstruction was found to vary in proportion to tubular driving force (P) the difference between intrarenal pressure in the cortex and ureteral pressure (Kiil et al Am. J Physiol 223: 1263-1270 1972) Further examinations on unblocked kidneys (Ostrik et al) showed that tubular dilatation on account of a rise in tubular transmural pressure was the main tubular hydrodynamic event during saline infusion; the rise in P could only account for a doubling of urine flow In other studies (Bader et al) the hemodynamic mechanisms contributing to passive changes in proximal reabsorption were investigated by injecting saline at high and control perfusion pressure in dogs At renal perfusion pressure of 170 mm Hg saline injection abruptly increased renal blood flow intrarenal pressure sodium excretion and urine flow whereas the responses were smaller at 110 mm Hg and barely perceptible at 80 mm Hg GFR was essentially constant at perfusion pressures exceeding 110 mm Hg and the highest natriuretic effect at high perfusion pressure therefore a consequence of tubular inhibition The exaggerated natriuretic response on acute elevation of renal perfusion pressure can be explained in terms of the autoregulation hypothesi of salt excretion (Kiil et al Acta physiol scand 76: 24-39 1969 Kiil Scand J clin Lab Invest 25: 113-117 1970)

34 Öberg, B Dept of Physiology University of Göteborg Sweden: THE INFLUENCE OF CARDIOVASCULAR RECEPTORS ON EXTRACELLULAR FLUID DISTRIBUTION AND RENAL WATER AND SALT EXCRETION

Cardiovascular receptors, sensing the fullness and the prevailing performance of the cardiovascular system have been assigned important roles in the control of extracellular fluid volume (e g Gauer Henry and Behn 1970) This volume regulation includes both the internal distribution of available extracellular fluid between intra- and extravascular compartments and the external fluid exchange with the environment primarily by affecting the renal handling of salt and water

Redistribution of fluid between intra- and extravascular compartments by way of the capillary filtration absorption process is effected by reflex resetting of the pre/postcapillary resistance ratio leading to appropriate shifts in the capillary hydrostatic pressure Such reflex adjustments of the resistance ratio result primarily from removal of the inhibitory restraint from the arterial baroreceptors and are most pronounced in the skeletal muscles which due to their *large mass constitute the most important extravascular fluid depot* in the organism (Öberg 1964) Through this mechanism the plasma volume can be maintained at the expense of the interstitial fluid content

Assuming that a quantitative relationship normally exists between the fluid contents of the intravascular and the total extracellular compartments a disturbance in the total extracellular fluid volume should influence cardiovascular receptors located mainly in the low pressure part of the circulatory system e g in the heart The efferent pathways of volume regulating reflexes from cardiac receptors include both hormonal mechanisms e g the release of ADH and neuronal mechanisms e g the renal vasoconstrictor fibres with consequent reflex influences on renal hemodynamics renin release and on tubular function - Stimulation of atrial receptors by atrial distension is said to inhibit ADH-release while the effect on renal circulation is rather antagonistic in nature i e a reflex vasoconstriction - *Ventricular receptors sensing the extent of ventricular* diastolic filling may also inhibit ADH-release and are capable of inducing particularly marked inhibitions of renal vasoconstrictor fibre discharge (Öberg and Thorén 1973) implying that the hormonal and circulatory responses complement each other in a synergistic way Reflexes from ventricular receptors may therefore constitute an important volume regulating mechanism.

Gauer O H J Henry and C Behn, Annual Review of Physiology 1970 32, 547 595
Öberg B Acta physiol scand 1964 62 Suppl 229
Öberg B and P Thorén Acta physiol scand 1973 In press

Björklund, A , Moore R Y and Stenevi U (Department of Histology University of Lund Sweden and Departments of Neurology and Anatomy University of Chicago U S.A): STUDIES ON MORPHOLOGICAL PLASTICITY OF CENTRAL ADRENERGIC NEURONS 25

The functional plasticity of the central nervous system is well known A morphological correlate of such plasticity would be a modifiability of axonal connections and in a series of studies we have explored whether intact catecholamine axons will show alterations in response to a removal of a non-adrenergic input to a terminal region

Two experimental situations were studied: the adrenergic innervation of the medial and lateral septal nuclei after removal of the non-adrenergic hippocampal afferents to these nuclei and the adrenergic innervation of the lateral geniculate body after removal of either the non-adrenergic retinal afferents or the non-adrenergic cortical afferents to this region After removal of the hippocampal afferents to the septum there was a gradual substantial increase in the adrenergic innervation within 30 days after operation and this effect persisted up to at least 100 days The increase in the number of adrenergic fibres was accompanied by a parallel increase in noradrenaline reaching values of about 200 % of control at 30-100 days In the lateral geniculate body there was a strong increase in the adrenergic innervation at 13 days after visual cortex ablation (i e at a time before the reactive glial changes in the nucleus became prominent) No effect was seen after enucleation however

It is suggested that the observed effects are the result of a collateral sprouting of the intact adrenergic axons in response to the denervation and that the sprouting fibres might reinnervate synaptic sites vacated by the axonal degeneration produced by lesion If this interpretation is correct it indicates that in fact central adrenergic axons are capable of significant morphological plasticity

36 Zimmer, J (Anatomical Institute B University of Aarhus Denmark):
ABERRANT AXONAL GROWTH (SPROUTING) IN HIPPOCAMPUS

The afferent systems to the hippocampus and fascia dentata are distributed in characteristic and often exclusive laminae Changes in this laminar pattern are observed following primary unilateral removal of the perforant path fiber input (deentorhination) Rats from 5 to 30 days old had this operation performed The terminal fields of the remaining systems were studied after various survival times by the Timm method or following secondary lesions and silver-impregnation

In general the deentorhinated zones of hippocampus and fascia dentata were somewhat shrunken but occupied by abnormal projections of the intact systems A decrease in the capability of extension of these systems was observed with increasing age at the deentorhination

With the silver-impregnation method extension of the terminal fields of both the ipsilateral association systems and the commissural systems was found

The mutual mediolateral distribution of the extended fibers followed the normal pattern in fascia dentata The commissural fibers spared the most superficial parts of the deentorhinated zones here

The Timm method gave corresponding results but revealed furthermore an extension into the outer parts of fascia dentata of a system normally terminating at CA1/subicular transition This observation is of special interest as this particular part was devoid of commissural fibers Evidence of a supragranular location of mossy fibers was also found By the two methods no evidence was found of extension or intensification of the terminal field of septal fibers An induced acetylcholinesterase rich layer in fascia dentata did not completely correspond with any Timm s staining

The replacement of the perforant path by other systems is discussed in relation to earlier observations on related phenomena The possible underlying mechanisms are considered and a definition of the terms aberrant axonal growth rerouting and sprouting and their characteristics is proposed

Storm Mathisen J (Norwegian Defence Research Establishment 2007, Kjeller, Norway) INCREASE OF CHOLINE ACETYLASE (ChAc) AND ACETYLCHOLINESTERASE (AChE) IN STRATUM MOLECULARE FASCIAE DENTATAE FOLLOWING DEGENERATION OF THE PERPHORANT PATH 37

Previous observations suggested that ChAc activity in the molecular layer of fascia dentata may increase after lesions of the entorhino dentate fibres (Storm Mathisen Brain Res 1972 40 215) To study this the entorhinal area was severed from the hippocampal formation in rats (200 g) Serial horizonal cryostat sections were cut and of each 10 8 were freeze dried 1 stained for AChE and 1 silver impregnated (Fink Heimer) Degenerating terminals were distributed in the outer and middle zones of the molecular layer of area dentata corresponding to the extent of the lesion (Hjort Simonsen J comp Neurol 1972 146 219) There was an increased AChE staining according to the distribution of degenerating nerve terminals Samples were dissected from the various zones and assayed for ChAc(Storm Mathisen op cit) ChAc was increased by about 50% in the sites with maximum density of degenerating perphorant path terminals There was no increase in the zones without such terminals The increased activities were present at 8 days survival and lasted at least 3 months They were lost on transection of the septal afferents It is suggested that the changes may represent a "compensatory" invasion of the vacant synaptic sites by cholinergic nerve terminals Similar results have been reported for AChE by Lynch et al (Brain Res 1972 42 311)

38 Andersen P Teyler T and Wester K (Institute of Neurophysiology University of Oslo Oslo Norway) LONG-LASTING CHANGE OF SYNAPTIC TRANSMISSION IN A SPECIALIZED CORTICAL PATHWAY

The large pyramidal cells of the hippocampus (CA3) have axons with two large branches One branch runs in the fimbria and contacts septal and hypothalamic cells The other branch curves back into the hippocampal formation proceeds through the CA3 region and forms a thin layer in the superficial part of stratum radiatum of CA1 In serial electron micrographs the latter fibres the Schaffer collaterals have Ranvier nodes with an average internodal length of 75 micron Most of the Ranvier nodes in CA3 and the adjoining part of CA1 issue 1-3 unmyelinated branches With intervals of 3-5 micron the unmyelinated fibres show boutons en passage most often contacting dendritic spines but also shafts of small dendrites With increasing distance from the CA3 the Schaffer collaterals lose their myelin and proceed as unmyelinated fibres with frequent synaptic swellings

All 165 CA3 cells tested were antidromically invaded from the fimbria as well as from the Schaffer collaterals in CA1 Both orthodromic and antidromic activation of the Schaffer collaterals gave synaptic waves at depths appropriate with the level of these fibres The size of the synaptic waves remained fairly constant throughout the distribution of this fibre system indicating that the number of CA1 cells per area which were synaptically activated remained relatively constant

In contrast to the behaviour of the fimbrio-septal pathway the Schaffer collateral-CA1 pathway showed marked frequency potentiation with an optimal frequency around 10-15/second Responses to single orthodromic volleys along the Schaffer collaterals showed long-lasting (several hours) increase following repeated hort tetani (10/sec for 15 sec) to the same pathway The threshold of the input was not changed but its steepness was increased indicating that a given volley was able to evoke a larger synaptic potential than before the tetanic priming These plasti changes may be related to the peculiar syn ptic arrangement allowing amplification of the effect of impulses along the Schaffer collaterals By varying the firing frequency the CA3 cells may thus bring larg r or smaller amount of CA1 neurones to discharge but retaining a relatively constant influence over the fimbrio-septal pathway

Johansen K Dept of Zoophysiology Århus University Denmark 39
ADAPTATIONS TO ENVIRONMENT OF CARDIOVASCULAR FUNCTIONS

Blood flow to tissues interfacing the vertebrate animal from its environ ment i gills lungs and skin will be reviewed in a comparative phylogenetical context emphasizing control of ventilation-perfusion matching and skin circulation in the transition from water bre thing to air breathing Fish gills r ceive a cardiac output (C O) ranging from 5 to 40 ml/kg min depending on species temperature and activity At normoxia O_2 uptake varies directly with (C O) Gills possess non-respiratory shunts influencing the effectiveness in O_2 uptake Shunt circulation seems to be under adrenergic control while gas exchange circulation is cholinergic Hypoxia incr ases gill vascular resistance yet the PO_2 gradient from water to blood decreases showing improved convection of blood and water Heart rate is coupled to ventilatory rate by a vagal r flex mechanism Ventilation-perfusion ratios are high (10-50) due to high ventilation neces itated by the low O_2 content of water Swimming altering distribution of water acro s gill causes in creased blood flow to well ventilated gills and reduced flow to gill with low ventilation Fi h myocardium shows higher tolerance to anoxic stress in bottom dwelling species than in species from better aerated water (Poupa & Gesser (pers comm)) Fish using bimodal breathing aquatic and aerial show reflex coupling of ventilation and perfusion of respiratory organs Air breathing increases C O and shifts blood flow from gills to air breathing organ The response is elicited by vagal tretch sensitive receptors in the air breathing organ In facultative air breathing fish (Amia) tempera ture increase will caus switching from water bre thing to air breathing Whil C O increases linearly with temperature gill vascular resistance drops precipitously when air breathing starts and low resistance bypas of the gills open up The hange is dependent upon a vagal sensory link Inflation dependent perfusion increase to the lung is p esent in anuran amphibia and reptile Stimulation of pulmonary vagal stretch receptors greatly lowers pulmonary vascular esistance increases C O and causes a left right shunt in snak s During long r spiratory pauses in aquatic reptiles an in creasing right to left shunting occurs Such shunting causes a matching of perfusion to O_2 availability in the lung

In aquatic and amphibious specie skin circulation i important in gas exchange Both fi h living in O_2 deficient waters and amphibians show high density f cutaneous capillaries Vasomotor mechanisms (neural and direct) cause skin vasodilatation under various conditions e g hypoxic wate (fish) prevention of air breathing (lungfish) CO_2 breathing pulmonary obstruction and diving (amphibians) In reptiles skin circulation i l ss important in gas exchang but has become important in temperature regulation It has been shown that skin blood flow (lizards turtles) increase during heating and decrease during cooling independent of body temp (B T)if the l tter is below preferred B T A right left shift and increased heart rate and sympatheti muscl vasoconstriction ar factors causing increased skin flow during heating The importance of skin circulation in thermal homeostasi is crucial in mammals and birds possibly maximal in birds from polar regions Skin of birds feet (Macronecte gigantsus) shows peak blood flows >200ml/kg min promptly onset on cold immersion Skin vasoconstriction and dilatation can be minicked by stimulation of cut peripheral nerves suggesting presence of neurogenic vasodilator mechanism Skin vascular smooth muscle shows adaptive change in sensitivity to vasoactive agents at lowered tissue temper ture

40 Hyvärinen J (Institute of Physiology, University of Helsinki, Finland): FUNCTIONAL PROPERTIES OF CELLS IN THE PARIETAL SOMATOSENSORY AND ASSOCIATION CORTICES OF THE MONKEY

Previous studies of the primary somesthetic cortex (S I) in anaesthetized monkeys have indicated a mediolateral somatotopic progression of receptive fields and a segregation of modalities such that neurons with cutaneous receptive fields are more common in the anterior part of the postcentral gyrus, and neurons of deep modality are more common posteriorly In awake behaving monkeys we have confirmed these findings, but we have also seen evidence for convergence of different modalities to cells that form cortical columns in the posterior part of S I and the parietal associative area (Brodmann's area 7)

Awake, sitting stumptail monkeys were studied with the transdural microelectrode recording technique developed by Evarts Peripheral receptive fields in the hand area of S I were mapped using tactile stimuli and manipulation of joints and muscles

Cells with simple skin fields were the majority of those studied in all the three cytoarchitectual areas of S I (Brodmann's areas 3, 1 and 2 from anterior to posterior) However, in a sample of 581 histologically localized cells studied in S I at the time of writing this abstract, the percentage of simple skin field neurons decreased from 70 % in area 3b to 63 % in area 1 and 33 % in area 2 Further posteriorly in the parietal area 7 this percentage was only 11 Whereas the number of simple skin fields decreased posteriorly, the number of more complex receptive fields increased in the same direction Cells that required a particular direction of movement along the skin, a particular orientation of a tactile stimulus edge or that were activated both from skin receptors and deep (joint or muscle) receptors were only 10 % of the total in area 3b In area 1 their number was 18 %, in area 2 it was 35 % and in area 7 they appeared to constitute well over half of the cells, but in that region the cells receive visual input, too These proportions must be considered tentative until more precise methods for identification of the input receptors are used, but these percentages show a gradual change from simple receptive fields anteriorly in S I to more complex activating connections in posterior S I and adjacent parietal associative area

The most prominent difference between S I and the adjacent parietal associative area is the visual activation in area 7 which we have not seen in S I The visual activation in area 7 is related to eye movements in a particular direction or to a particular direction of gaze Moreover, manipulative movements of the hand in the same direction often activate cells in area 7 also when vision is blocked Thus cells in this region are truly "associative" between two different modalities, being activated by both somesthetic and visual mechanisms related to a particular direction in the space around the animal

This work has been supported by the Foundations' Fund for Research in Psychiatry

Folkow B Dept of Physiology Univ of Göteborg Sweden: FROM PHYSIOLOGY TO PATHOPHYSIOLOGY - PRIMARY HYPERTENSION A DISORDER OF REGULATION 41

Although primary (essential) hypertension has initially a circulatory pattern similar to a mild defence reaction its established phase is characterized by a raised systemic flow resistance It has been widely assumed that this increased resistance is due to a raised vascular smooth muscle activity Consequently the intense study of the pathogenesis of this common disorder of regulation has concentrated on the origin of this increased smooth muscle tone The debate has centred inconclusively on neurogenic hormonal and ionic factors and/or on an increased effector sensitivity to such influences

The problem has been approached from an entirely different angle in studies based on haemodynamic analyses in man and spontaneously hypertensive rats (SHR) These results (see Folkow 1971) strongly indicate that in both types of primary hypertension the raised resting resistance is essentially the result of an increased arteriolar media thickness partly encroaching upon the lumen even at maximal dilatation This implies a normal structural adjustment to increased functional load similar to hypertrophy of the left ventricle and of arterial walls in general (cf Suwa and Takahashi 1971) Thus even at maximal dilatation precapillary resistance is raised above control values Moreover for given smooth muscle activations exaggerated luminal reductions ensue resulting in a raised resistance and vascular hyper reactivity even at normal levels of smooth muscle activity Also the important baroreceptor re-setting (cf Aars 1969) appears to be mainly a consequence of such a vascular wall adaptation

Studies on rats show that this structural adaptation is such a rapid process that it becomes closely interwoven in time with functional triggering factors which in SHR are at least partly constituted by the neurohormonal excitatory pattern commonly induced by increased alertness or alarm (the defence reaction) When often repeated such reactions lead to a gradual rise in resting pressure even in rats that are not genetically predisposed to hypertension The greater the average pressure load the stronger is the tendency for structural adaptation of the heart and precapillary vessels Comparisons between SHR and normotensive and renal hypertensive rats suggest that SHR display exaggerated defence reactions to standardized stress situations and further that their heart and vessels are somewhat more prone to adapt structurally to a given pressure presumably forming part of the polygenetic background of their hypertension Parallels to man suggest that similar principles might contribute to the hereditary background of essential hypertension as reinforced by the extrinsic drive constituted by stressful environmental influences - Hypotensive treatment by drugs or other interferences can cause rapid regression of the structural changes and may when used e g for prevention in prehypertensive SHR largely hinder the development of both hypertension and structural vascular changes

Ref Folkow B Clin Sci 1971 41 1 12

Suwa N and T Takahashi Morphological and Morphometrical Analysis of Circulation in Hypertension and Ischemic Kidney Urban & Schwarzenberg München-Berlin-Wien 1971

Aars H Acta physiol scand 1969 75 406-414

42 Vallbo, Å,B (Department of Physiology Biological Institute University of Umeå Sweden); THE ROLE OF THE MUSCLE SPINDLES IN VOLUNTARY MOTOR ACTIVITY

The discharge from muscle spindles in the antebrachial finger flexor muscle of conscious human subjects was analysed when the muscles were relaxed during isometric voluntary contractions and during active movements The spindle discharge increased with muscle length in relaxed muscles and was largely constant with time after an adaptation period of 5-15 sec At a comfortable resting position of the hand only five percent of the spindle primaries were continuously discharging their impulse frequency did not exceed 20 ips (impulses per sec) and their sensitivity to muscle length was below 1 0 ips/mm The low discharge rate and the low sensitivity in comparison with other preparations indicate that the fusimotor outflow is negligible to relaxed muscles and any functional role of the poor afferent activity under these conditions may be questioned altogether No dramatic changes in spindle afferent discharge could be provoked by noxious or non-noxious skin stimuli by varying the input through the neck reflex afferents or to the vestibular organs as long as the muscles were relaxed Thus there were no functionally significant modulations of the fusimotor activity in this preparation by a number of procedures which have been shown in animal experiments to modify the fusimotor outflow

During voluntary contractions on the other hand there was a powerful fusimotor outflow which largely ran parallel with the skeletomotor outflow in spatial temporal and intensive respects This implies somewhat unexpectedly that the basic pattern of intrafusal activation is very similar in the human hand and in the much more primitive system of amphibia the design of which entails an obligate and rigid co-activation and parallelism of the extrafusal and intrafusal muscle activity This suggests that the significant role of the separate fusimotor system in higher animals is to refine the performance rather than to alter the basic function of the muscle spindle system

A closer analysis of the temporal relations at the onset of voluntary contractions revealed that the contractions were all initiated by the skeletomotor neurones whereas the rest of the contractions involved the joint activation of the two motor systems in fast as well as in slow contractions This shows that the command signal to the muscle is not delivered to the gamma motor neurones alone and therefore the follow up length servo hypothesis must be rejected

During active movements many spindle primaries signalled the speed of movement quite accurately The reflex effects of this afferent activity on the spinal level would be to eliminate variations in the speed of movement and thus to make the active movement progress more smoothly and fluently Although this function may represent but a limited facet of the role of the muscle spindles the findings suggest that the main significance of this complicated system is during contraction and in regulating the characteristics of active movements

Siesjö, B.K. (Brain Research Laboratory University Hospital Lund Sweden): REGULATION OF CEREBROSPINAL FLUID pH. **43**

Recent results demonstrate that the pH of bulk cerebrospinal fluid (CSF) equals that of the cerebral extracellular fluid (ECF) Since the ECF pH serves as an important regulator of pulmonary ventilation and a major determinant of cerebral blood flow many attempts have been made to unravel the physiological mechanisms which are responsible for the control of the CSF pH. These mechanisms still remain largely unknown and several possible regulatory factors will therefore be described and examined

The CSF (and ECF) is a bicarbonate-containing fluid which contains very little protein or other non-bicarbonate buffers Its buffer capacity to CO_2 is therefore low and the HCO_3^- concentration does not vary significantly when the CO_2 tension is altered in vitro It follows from this that variations of CSF HCO_3^- concentration in vivo are due to ion exchanges with either blood or tissue cells

At steady state and under normal acid-base conditions the CSF HCO_3^- concentration and the CSF pH are lower than in plasma water This relative acidosis in CSF is maintained in spite of the fact that the d c potential between CSF and plasma is positive (about 4 mV at a plasma pH of 7 40) The net electrochemical potential differences for H^+ and HCO_3^- ($\Delta\ \mu$) cannot be explained by the fact that the CSF is secreted as an acid fluid. In fact the results obtained by Maren suggest the opposite i.e that the newly formed CSF contains a high bicarbonate concentration There are two possible mechanisms which could maintain the net $\Delta\ \mu$ for H^+: (1) active transport of H^+ from plasma to CSF or (2) efflux of acids from the tissue cells

In respiratory acidosis there are relatively rapid increases in the CSF HCO_3^- concentration which limit the fall in pH. The mechanisms involved cannot achieve complete normalization of the CSF pH but operate even if the plasma HCO_3^- concentration is kept constant In nonrespiratory acidosis and alkalosis the CSF HCO_3^- concentration varies much less than the plasma HCO_3^- concentration even in chronic cases and the CSF pH shows a remarkable constancy Calculations of electrochemical potential differences show that the $\Delta\ \mu$ values are essentially unchanged and thus give littl support to the hypothesis of an active transport regulation of CSF pH. The CSF/plasma d.c potential varies inversely with the plasma pH even in chronic acid-base changes and it remains a pos ibility that the regulatory changes in the HCO_3^- ratio between CSF and plasma are at least partly caused by the changes in d c. potential.

Hyperventilation elicited by hypoxia is accompanied by an efficient regulation of the CSF pH. However if one assumes that the hypoxia leads to increased acid production in the brain the pH homeostasis may reflect respiratory regulation of a primary acidosis of nonrespiratory nature In nonrespiratory acidosis and alkalosis an efficient regulation of the CSF pH occurs only when a compensatory change in pulmonary ventilation can occur Since neither respiratory acidosis nor passive hyperventilation is accompanied by complete or near-complete restitution of the CSF pH, it appears that the pronounced regulation of the pH only occurs in nonre piratory conditions that allow ventilatory adjustments

44 Thesleff S. (Department of Pharmacology University of Lund Sweden):
CHOLINERGIC RECEPTORS IN MAMMALIAN SKELETAL MUSCLE

In innervated mammalian skeletal muscle acetylcholine (ACh) receptors are present in great density about $10^4/\mu m^2$ at the end plate region while their density at extrajunctional sites is less than $5/\mu m^2$ Following denervation the receptor density at the end plate is maintained but that of the extrajunctional membrane is increased to about $10^2/\mu m^2$

When extrajunctional cholinergic receptors appear the rate of rise of the action potential is markedly reduced and the spike is followed by a long-lasting positive afterpotential At the same time the action potential becomes resistant even to high concentrations (10^{-6}-10^{-5}) of tetrodotoxin (TTX) The appearance of TTX-resistant action potentials coincides spatially and temporally with the increase in the number of extrajunctional cholinergic receptors

The induction of extrajunctional cholinergic receptors and of TTX resistant action potentials in denervated muscles is prevented by the administration of actinomycin D to the animal However once the denervation changes are established actinomycin has no effect on these membranes properties indicating that their induction depends on genetically induced protein synthesis

In view of the temporal and spatial correlation between the appearance of extrajunctional cholinergic receptors and of TTX resistant action potentials it has been speculated that structural proximity or identity between the two types of ionophores may exist in the denervated muscle membrane

The application of colchicine or vinblastine drugs known to block axoplasmic flow to the sciatic nerve of the rat induce the appearance of extrajunctional cholinergic receptors and of TTX resistant action potentials in leg muscles This occurs without affecting nerve impulse transmission no paralysis of the hind leg is observed and the twitch as well as the tetanic tension of the muscle is intact Neither is spontaneous transmitter release affected by the drugs It is concluded that lack of substances normally carried by axoplasmic transport induce the appearance of extrajunctional cholinergic receptors and of TTX resistant action potentials in denervated muscle This conclusion is supported by the observation that the time of onset of the denervation changes depends upon the length of the nerve stump distal to the site of neurectomy

Ussing, H H (Institute of Biological Chemistry A University of Copenhagen Denmark) TRANSPORT PATHWAYS IN EPITHELIA 45

When studying the transport of solutes and water across epithelia one can use the black box approach and describe the processes in terms of active transport of certain substances which in turn by electric or hydraulic coupling may contribute to the transport of passively transported substances

In recent years however the interest has been switched towards a resolution of the generalised fluxes across the epithelia into flows through specific pathways

It has become apparent that epithelia can be of a high resistance and a low resistance type Formally the epithelia of the latter type (e g gall bladder small intestine proximal kidney tubule) differs from those of the former type (frog skin urinary bladder large intestine etc) in that they possess a pronounced shunt pathway which is rather indiscriminately permeable to small ions and small hydrophilic molecules Hyperosmolarity on the mucosal side of several tight epithelia transforms them reversibly into the leaky type Evidence coming from several laboratories including our own indicate that the main shunt pathway in the leaky or low resistance epithelia is intercellular through "leaky seals The leaky seals have been visualized for instance by precipitation of $BaSO_4$ (Ussing H Phil Trans Roy Soc Lond B 1971 262 85) or lanthanum (Martinez-Palomo A , Erlij D and Bracho H J Cell Biol 1971 5o 277) or they have been localized electrically for instance by probing the surface of the epithelium with a micro electrode (Frömter E and Diamond J Nature 1972 235 9)

Another type of pathway is provided by the coupling between neighbouring cells through low resistance cell contacts Present data indicate, however that in the case of say frog skin the coupling between cells in different layers of the epithelium if existent must be through rather high electric resistance paths This conclusion is based on the volume changes in different layers in response to electric current flow in the inward and outward directions (Voûte C and Ussing H Exp Cell Res 197o 61 133)

The polar behaviour of epithelia stems from two independent sources: 1) The fact that the tight or leaky seals are placed assymmetrically, mostly near apical end of the mucosal side of the epithelia and 2) the fact that the inward and outward facing cell membranes of the epithelium differ with respect to passive and active transport pathways

46 Lowy J (Institute of Biophysics Århus University Denmark)
THE STRUCTURAL BASIS OF CONTRACTION IN VERTEBRATE SMOOTH MUSCLES

The major difficulty in using the sliding filament model to explain contraction in vertebrate smooth muscles has been that here the presence of myosin in a filamentous form could not be established unambiguously This was achieved only recently by X-ray experiments with living muscles (for references see Lowy et al Phil Trans Roy Soc 1973 265 191) Such experiments also provided further evidence for the operation of a sliding filament mechanism by showing that during contraction the axial periodicities due to the arrangement of the actin and myosin molecules remain practically constant A number of important problems remain unsolved One of them is whether the filamentous myosin elements occur in the form of ribbons or rods Another concerns the arrangement of the actin and myosin filaments in the contractile apparatus Both problems are at present the subject of a lively controversy From their X-ray and electron microscope work Lowy et al believe that myosin is present in unique ribbons which are randomly disposed in the cell On evidence from electron microscopy Somlyo et al (for references see Phil Trans Roy Soc 1973 265 233) propose a structural picture which resembles more closely that seen in striated muscles namely rod-like myosin filaments arranged in a regular lattice

At present there are great difficulties in the way of solving the ribbon-rod problem We have concentrated on an X-ray technique that uses a high-resolution double-monochromator camera of the type described by Huxley & Brown (J Mol Bio 1967 9 383) This enables us to determine some of the dimensions the myosin elements in living muscles Results obtained by Lowy Poulsen & Vibert show that in the guinea pig s taenia coli muscle maintained in a cold hypertonic Ringer solution the myosin cross bridges are in register over a distance of about 1300 Å laterally (lateral coherent unit = LCU) In muscles kept in cold isotonic Ringer the LCU is less than 1300 Å but at least 800 Å These figures exclude the possibility of diffraction from individual rods which (according to Somlyo et al) have a diameter of at most 200 Å plus a halo region that surrounds each rod and extends to no more than another 200 Å But these rods were visualised by fixation of muscles in warm isotonic Ringer and from our X-ray results to date we cannot give a figure for the LCU of muscles kept under such conditions What we have established is that in cold hypertonic Ringer, myosin elements aggregate to form structures which are larger than those present in cold isotonic Ringer

We have as yet no evidence from studies of the equatorial diffraction pattern that the myosin elements (under any conditions) are organised in a regular sideways arrangement like the one seen in the electron micrographs of Somlyo et al

Carlsson A. (Department of Pharmacology University of Gothenburg Sweden): 47
CENTRAL AND PERIPHERAL MONOAMINERGIC MECHANISMS

Monoaminergic neurons maintain their transmitter stores relatively constant in spite of considerable variations in physiological activity suggesting that transmitter synthesis is promptly adjusted according to the needs Induction of the rate-limiting synthetic enzyme tyrosine hydroxylase occurs only after a latency of several hours The increased transmitter synthesis demonstrated after short-term stimulation is therefore presumably due to an activation of existing enzyme molecules It has been proposed that this short-term adjustment is mediated via changes in end-product inhibition, but such inhibition requires relatively high catecholamine concentrations (about 10^{-4} M) and is thus of doubtful physiological significance

Two examples will be given where short-term regulation of tyrosine hydroxylase activity in vivo occurs independently of release by nerve impulse and where end-product inhibition does not seem to be involved

1) In the adrenal medulla of the rat dopamine (DA) mainly if not entirely serves a precursor role constituting about one per cent of the total catecholamines Neurogenic stimulation of the gland by means of drugs acting via supraspinal centres causes a considerable increase in DA occurring in spite of a marked acceleration of DA turnover and thus presumably due to an increased synthesis caused by an activation of tyrosine hydroxylase Chlorisondamine a ganglionic blocking drug inhibits the release of adrenomedullary hormones efficiently but leaves the elevation of DA unaffected As indicated by turnover data this elevation is due to an increased synthesis The effect is abolished by transection of the spinal cord — Thus the tyrosine hydroxylase activity of the adrenal medulla can be promptly adjusted via a neurogenic mechanism operating at least partly independently of the physiological release mediated via nicotinic receptors

2) If the nigrostriatal DA axons of the rat are cut the striatal tyrosine hydroxylase activity in vivo is markedly elevated during the first 30-60 min This paradoxical response is presumably due to a feedback mechanism mediated via postsynaptic (or possibly hypothetical presynaptic) DA receptors Evidence for this hypothesis has been obtained by demonstrating changes in the tyrosine hydroxylase activity in vivo after the administration of DA eceptor agonist (apomorphine) and an antagonist (haloperidol) either separately or combined — It should be noted that this control mechanism is capable of operating in the absence of an impulse flow

These prompt and marked adjustments of tyrosine hydroxylase activity probably occur without any concomitant changes in the synthesis of macromolecular cell constituents and are thus difficult to reconcile with the concept that the release by nerve impulses occurs exclusively through exocytosis

48 Rosell, S. (Department of Pharmacology Karolinska Institutet, Stockholm Sweden): VASCULAR REACTIONS AND MOBILIZATION OF FAT IN ADIPOSE TISSUE

It is reasonable to assume that the blood circulation in adipose tissue is well adapted for the specific function of p oviding an effi cient transport of fat The adrenergic neuro humoral system seems to be of prime importance in the control of circulation and lipolysis in adipose tissue The lipolysis leads to an outflow of free fatty acids (FFA) and glycerol

From available data in dogs some characteristics can be established and compared with other vascular circuits as e g skeletal muscle Resting blood flow in adipose tissue is 6 9 ml/min/100g The maximal blood flow produc d by vasodilating agents (about 40 ml/min/100g) is less than the corre ponding value for skeletal muscle This seems to imply smaller dimensions of the arterial system in adipose tissue On th other hand resting and maximal values for the capillary fil tration coefficient (CFC) are larger than in skeletal muscle The den sity of th capillary network surrounding every adipocyte is exten sive In addition morphological studie indicate that the adipocytes at lea t in the mesentery are located toward the venous section of the capillary bed rather than to the arterial side This indicates that the adipocyt s are localized near ve sels with the highest permeabi lity These data indicat that favou able conditions for capillary ex change exist in subcutan ous adipose tis ue

Stimulation of the appropriate sympathetic nerve to canine subcu tan ous adipo e tissue produc s adjustments in all the series coupled s ctions of the vascula bed i e constriction of resistance and ca pacitance ve els and a net transcapillary fluid absorption A conspi cuous difference from such adjustments in other tissues is a marked inc ea e in CFC during stimulation sometimes to three times the ol valu In addition the isovolum tric capillary pressure (Pci) reases Th s data indicate that increased neuro humoral activity may increase the capillary permeability Thi inc ea e is concomi tant with a d cr ased capillary urface area as indicated by a dimi nish d disappearance of xenon from a local tissue depot despite a con tant total blood flow The d scribed adjustments of th capillary bed may p omote the outflow of the product of lipoly is without a simultaneous outward fluid filtration

The lipolytic rate is enhanced by increased neuro humoral activity Qu ntitative compa isons indicate that the innervation of adipose tissue i more important for the control of the lipolysis than the circulating catecholamines However th r are regional diffe ences in th im portance of the sympathetic neu o humo al control of lipolysis Per haps hormones ather than the sympathetic system play a primary rol in some regions Thi possibility has not yet been evaluated x perimentally

Lundgren O Dept of Physiol Univ of Göteborg Sweden: CIRCULATORY FACTORS IN INTESTINAL ABSORPTION 49

Intestinal blood flow represents one factor of importance for intestinal absorption in vivo, although blood flow in the mucosal layers has been considered to be normally so large as to never limit the rate of intestinal absorption However the demonstration of a countercurrent exchanger in the intestinal mucosa has greatly complicated the seemingly simple relationship between blood flow and absorption Anatomically this countercurrent exchanger is constituted by the arterial supply to each villus as surrounded by the dense subepithelial capillary network in which flow is countercurrent to arterial inflow The distance between the two limbs of these hairpin vascular loops of the villi is only 10-20 m This vascular arrangement has functional implications that will now be briefly summarized (cf Lundgren Acta physiol scand. Suppl 303)

Absorption of easily diffusible lipophilic solutes Mean transit time of plasma particles in the villous vascular loops amounts to 3 5 sec at resting blood flow in cat small intestine 9/10 of this time being spent in the subepithelial capillary network Since most lipid soluble substances equilibrate almost completely in a fraction of a second between the two vascular limbs of the countercurrent exchanger absorbed lipophilic solutes may easily diffuse from capillary to arterial vessel along the concentration gradient established upon absorption The solute is then brought back towards the tip of the villus and hence hindered" from being absorbed Such a delaying effect on intestinal absorption has been demonstrated in model experiments studying ^{85}Kr-absorption (Svanvik Acta physiol scand Suppl 385)

Absorption of fatty acids It is wellknown that long chain fatty acids are absorbed mainly via the lymph while short chain ones are transported mainly in the portal blood These different absorption routes have been explained in terms of a rapid esterification in the epithelial cells of long chain but not of short chain fatty acids The partition between lymph and blood for different fatty acids may however also be explained by the countercurrent exchanger acting as an efficient hindrance to net blood absorption of mainly the lipophilic long chain fatty acids in a way similar to that described above for other lipophilic solutes These trapped" fatty acids are then eventually built into triglycerides in the epithelial cells The water soluble short chain fatty acids on the other hand are far less easily affected by the exchanger since they are pore-bound when passing the vascular endothelium Recent experimental observations strongly support this hypothesis (Jodal The significance of the intestinal countercurrent exchanger for the absorption of sodium and fatty acids Göteborg 1973)

Absorption of sodium and water The intestinal absorption of solutes and water is closely linked and it is assumed that a difference in osmolarity across the intestinal epithelial barrier is a prerequisite for the transepithelial transport of water This hyperosmolar region is according to current theories localized to the epithelial intercellular spaces However recent findings favour the view that the villous countercurrent exchanger functions as a countercurrent multiplier during absorption of e g sodium chloride creating a interstitial hyperosmolarity which in the villous tips reaches at least 600 mOsm/l This hypothesis also implies that the initial water absorption to a large extent takes place via the villous lymphatics

50 Mellander, S (Institute of Physiology University of Lund Sweden): OSMOLAR CONTROL OF THE CIRCULATION

Osmolar changes in the organism regional as well as more generalized ones can affect cardiovascular hemodynamics profoundly via marked influences on vascular tone fluid distribution between compartments and plasma volume Osmolar control mechanisms seem of special importance for the circulatory adjustments in muscle exercise and in certain pathophysiological states such as hemorrhagic hypotension

In exercise pronounced tissue hyperosmolality develops in the interstitial space of active muscle due to production and release of osmotically active particles from the contracting striated muscle fibres The interstitial hypertonicity leads to osmotic shrinkage of the vascular smooth muscle cells and to changes in transmembrane ionic concentration gradients and membrane permeabilities which cause inhibition of vascular tone: Tissue hyperosmolality acts thereby as one of the dominating mediators of functional hyperemia in muscle and it also improves capillary flow distribution and exchange in the tissue (Mellander S et al Angiologica 1967 4 310; Lundvall J Acta physiol scand 1972 suppl 379) A similar mechanism may contribute to the vasodilation in the myocardium and sweat glands during work - Exercis further leads to a very rapid exudation of plasma fluid int the xtravascular space of the active muscles mainly (> 75 %) due to osmosis caused by the work-induced tissue hyperosmolality but also to filtration caused by increased capillary pressure In heavy short-term whole-body exercise in man more than 1 litre of plasma fluid is lost to the active muscles Y t the plasma volume reduction in the exercise period is kept within tolerable limits owing to concomitant absorption of some 500 ml of extravascular fluid from inactive tissues This compensatory fluid g in is partly caused by osmosis due to a pronounced generalized arterial hyperosmolality which results from overflow of osmole from the active muscle mass and is partly caused by a reflex decrease of capillary (Lundvall J t al Acta physiol scand 1972 85 258) The erial hyperosmolality in exercise also seems to promote the action of heart via an inotropic effect (Wildenthal K et al Amer J Physiol 216 898) and to decrease pulmonary vascular resistance and concomitantly constrict the pulmonary veins so as to mobilize blood for the systemic circul tion (B4 G et al Acta physiol scand 1971 82 375) The r flex increas of blood pressure and the tachycardia in exercise in part may be ttributed to stimul tion of local osmoreceptors in active muscle (Lasser R. et al Circulat Res 1960 8 913)

In hemorrhag there is also an important compensatory osmolar control of plasma volume vi transcapill ry fl id absorption caused by pronounced arterial hyperosmolality In this case however th arterial hyperosmolality is rel ted to hyp glycemia caused by glucos release from the liver (Järhult J et l Acta physiol scand 1972 85 142)

Fluid escape int the extravascul r space seems inevitable when tissue hyp rosmolality dev lops It occurs besides in active muscle in glands during s cretion and in certain p thophysiological states such as second degree burns (Arturson G and Mellander S Acta physiol cand 1964 62 457) *Fluid movement* i th i te stitial spac may help to improve tissue nutrition sin e s ch fluxes have been found to augment the capillary transfer of sol t s (Lundgre O and Mellander S Acta phy iol scand 1967 70 26)

Irvine D R F [1) above] and Wester K G [2) above] Department of Psychobiology University of California Irvine): MIDDLE EAR MUSCLE EFFECTS ON COCHLEAR RESPONSES TO BONE-CONDUCTED SOUND 51

Contractions of the stapedius and tensor tympani muscles were elicited by electrical stimulation of their motor nerves or of the muscles themselves in anaesthetized cats The effects of these contractions on cochlear microphonic responses to air- and bone-conducted sound were examined Stapedius contractions that produced changes in air conduction similar to those observed under physiological conditions had almost identical effects on bone-conduction Tensor tympani effects on bone-conduction were of similar magnitude but greater complexity than those on air and varied as a function of the location of the bone-conductor on the skull Control observations established that the effects were attributable to the middle ear muscles and not to other consequences of the experimental procedures and that they did not reflect modification of an air-conduction component of the bone-conduction stimulus The functional significance of these effects is discussed in terms of protection against masking of environmental sounds by self-generated bone-conducted sound It is concluded that the middle ear muscles may subserve such a protective function

1) Present address: Department of Psychology Monash University Clayton Victoria 3168 Australia
2) Present address: Institute of Neurophysiology University of Oslo Karl Johansgt 47 Oslo Norway

Sovijärvi A R A, (Institute of Physiology, University of Helsinki, Finland): DETECTION OF TEMPORALLY AND SPATIALLY COMPLEX ACOUSTIC SIGNALS BY CELLS IN THE CAT PRIMARY AUDITORY CORTEX 52

The neural mechanisms involved in detection of complex and moving sounds were studied by recording single-neuron responses from 132 cells in the cat primary auditory cortex The cats were paralyzed and under neuroleptanalgesia The responses evoked by pure tones and by many types of natural complex sounds were compared with each other 25 cells mainly giving inhibitory and phasic responses to pure tones were selected for tests with moving sounds Excitatory, inhibitory and phasic components in the pure tone responses were almost equally common Phasic-response cells generally reacted in a time-locked fashion to transient complex sounds as could be predicted Non-predictable responses to complex sounds were given by 32 per cent of the cells; some cells responded in quite a different way (e g excitatory contra inhibitory) to complex sounds than to pure tones, and some others (17 %) responded only to certain complex sound patterns From 25 cells tested with moving sounds 8 responded selectively to the direction of the movement of the sound source The findings indicate that the primary auditory cortex contains cells, which may have a specialised function in detection of complex sound structures and the direction of sound source movement

53 Van Essen D , and Kelly, J (Department of Neurobiology Harvard Medical School Boston Mass USA) STRUCTURAL AND FUNCTIONAL PROPERTIES OF SINGLE CELLS IN AREA 17 OF THE CAT

Most neurons in area 17 of the cat can be classified as stellate or pyramidal on morphological grounds and as simple complex or hyper complex on the basis of their responses to visual stimuli We have used the technique of intracellular dye injection to study the relation ship between the anatomy and physiology of these cells Microelectrodes filled with Procion yellow were used to stain single neurons whose receptive fields were mapped with spots and slits of light Forty nine cells have been successfully injected and identified Most of the simple units were stellate (8 of 12) while pyramidal cells constituted the majority of complex cells (19 of 28) and hypercomplex cells (5 of 7) One cell with intermediate functional properties was pyramidal Several of the injected complex cells were neither stellate nor pyramidal but belonged to more infrequently occurring neuronal types such as multiform and double bouquet cells Simple cells occurred most frequently in layer IV of the cortex complex units were aggregated in both superficial and deep laminae and hypercomplex cells were concentrated in layers II and III These results indicate that there is a close correlation between the structure and function of individual neurons in the visual cortex (Supported by NIH Fellowships NINDS 5 F02NS51986-01 and NEI 1 F02 EY51537-01 and NIH Grant Nos 5 R01 EY00605 13 and 5R01 EY00606 08)

54 Poranen, A, (Institute of Physiology, University of Helsinki, Finland): MULTIPLE UNIT AND EEG ACTIVITY IN DIFFERENT SOMATOSENSORY AREAS OF BEHAVING MONKEYS IN A VIBRATION DETECTION TASK

The roles of different structures in the somatosensory system various somatosensory tasks have remained equivocal in spite many ablation studies Therefore, multiple unit and EEG recordings were made in different somatosensory areas of behaving monkeys taught to attend to a cutaneously presented vibratory signal or to ignore it

In the VB complex of the thalamus vibration causes a clear response which is sometimes modified by attention The cell groups in areas 3, 1 and 2 responded well to vibration but attention had only slight effects Vibration also activated parts of S II and motor cortex and sometimes attention had an effect, too In area 7 there were found no responses to vibration In all areas mentioned there were cell groups which did not change their response with attention

The results indicate that attention to vibration can modify the activity of some cell groups in different somatosensory areas Thus these areas are obviously participating in the sensory-motor interaction that takes place during the performance of the task The numerous recordings in which attention had no effect on the cellular activity indicate that the interaction may occur on lower levels of the central nervous system, too

Edwall L, G Ha gerstam and L Olgart (D partment of Pharmaco logy Karolinska Institutet Stockholm Sweden): EXCITATION OF IN TRADENTAL SENSORY UNITS AND CHOLINERGIC RECEPTORS 55

Physical stimuli such as air blasts drying and reduced pressure applied on exposed dentin in human teeth produc pain. These stimuli also give rise to afferent nerve activity from the teeth of the cat. Morphological studies have shown th presence of cholineste ase in senso y ne ves in the dental pulp as well as structure resembling a synaptic cleft between senso y nerve endings and odontoblasts (Arwill & Lilj Oral Physiol. Pe garron Press 1972 Arwill Odont.Rev 1967 18 191) Thi l ad to the question of whether or not acetylcholine is a mediator in dental pain transmi sion upon physical stimuli

N rve impuls s from the pulp were recorded by m an of low impe dance electrodes in e ted in dentinal cavities in the tooth of the cat (Edwall & Scott Acta physiol.scand 1971 82 555) An air blast proved to be the most efficient physical timulus to excite the intradental n r ves Local application of acetylcholine caused a simila e pons This response to acetylcholine was followed by a t ansient blockage to r pe ted application. Th espons to acetylcholine could be blocked by d tubocurarine atropine and hexamethonium administe ed locally In cont ast, the re ponse upon physical stimuli could not be blocked by the anticholinergic drug Moreov r afte application of acetyl holin and during the period of depression to acetylcholine the p epa ation r sponded to physical stimuli. These finding sugge t that ace tylcholine i not a mediato in the int adental pain transmission upon physical stimuli.

Knibestöl, M. (Department of Physiology Biological Institute University of Umeå, Sweden): QUANTITATIVE STUDIES ON SLOWLY ADAPTING MECHANO-RECEPTORS IN THE HUMAN GLABROUS SKIN AREA. 56

It has recently been shown that two types of rapidly adapting mechanoreceptors in the human glabrous skin area (RA-receptors and PC receptors) have a stimulus response relationship that can be best described by a log hyperbolic tangent function and not by a simple power function as has generally been reported for cutaneous mechanoreceptors in many animal species (Knibestöl M. J Physiol 1973 to be published)

Stimulus-response functions have now been analysed for a number of slowly adapting cutaneous mechanoreceptors in the same area (type SA-I and type SA-II) The results seem to indicate that for these receptors it may be of importance what measure is used for the nervous discharge If the nervous discharge is expressed as the total number of nerve impulses evoked by a stimulus the stimulus response curves have a sigmoid shape with a lower *purely dynamic part and an upper static part In this case* the log hyperbolic tangent function is the best mathematical description of the curves over the total range of stimulus intensities whereas a power function is a reasonable description only for the static part of the curves If as a measure of the nervous discharge is used the mean impulse frequency of the sustained discharge the log hyperbolic tangent function is still the best description for the majority of units whereas the power function is the best description for a few units

It is concluded that stimulus response relations for human cutaneous mechanoreceptors in general are best described by non-power functions

57 Mustaparta, H (Max-Planck-Institut für Verhaltensphysiologie Seewiesen, W-Tyskland): THE OLFACTORY SENSE OF THE PINE WEEVIL (HYLOBIUS ABIETI)

The possibility of using olfactory attractants in controlling pest insects has increased the importance of investigating their olfactory sense The pine weevil causes great damage to European forests and the question is which olfactory attractants are involved in its orientation

Electron microscopical studies have revealed two main types of olfactory sensilla localized to different fields of the antennal club Each sensillum is innervated by one or two sense cells The function of these cells is studied electrophysiologically By recording extracellular spikes from single cells while stimulating with different odours up to 200 a response spectrum for each cell was obtained The most potent stimulating agents were found within naturally occurring mono- and bicyclic monoterpenes

The response spectra indicate that these two terpene groups interact mainly with their respective acceptor types Since these acceptor types are situated in different cells discrimination between those terpenes is assumed to take place on receptor level However overlap of response spectra for other cells within these substances suggest an additional central nervous evaluation in the discrimination between them The same seem to be the case for the bicyclic terpene pinene and the bark beetle pheromone frontalin which are two of the most important of the tested compounds with respect to response degree and specialization of the cells Preliminary behavioural observations indicate that these two substances are also important for the orientation of the animal

58 Døving F B (Institute of Zoophysiology University of Oslo) Nordeng, H (Zoological Laboratory University of Oslo) Oakley, B (Department of Zoology Michigan University Ann Arbor USA) Russwurm H (present adress Norwegian Food Research Institute Ås) SKIN MUCUS BASIS FOR ODOUR DISCRIMINATION IN MIGRATING CHAR (Salmo alpinus L)

Nordeng (1971) has provided evidence that migrating char locate their spawing ground by pheromone trails produced by the young river population Extracellular recording of single unit activity in the olfactory bulb of these fish demonstrates that different char populations evokes different reaction patterns Thus a physiological basis for the pheromone hypotheses is given Skin mucus of one fish most frequently evoked the same kind of responses as did water in which this fish had been swimming It therefore seems probable that the skin mucus contains the substances responsible for the physiological responses in the bulbar neurones In attempts to identify the active substances samples of skin mucus were fractionated in a Sephadex G 25 column with destilled water as effluent The fractions were tested on the fish while recording the gross bulbar activity Peak activity was obtained for one fraction only Different mucus samples gave peak activity with different fraction numbers

Nordeng H (1971) Nature 233 411-413

Lindvall O and A Björklund (Department of Histology University of Lund Lund Sweden): GLYOXYLIC ACID CONDENSATION A NEW FLUORESCENCE HISTO CHEMICAL METHOD FOR BIOGENIC MONOAMINES AND RELATED COMPOUNDS 59

Glyoxylic acid (GA) has a considerably higher capacity than formaldehyde (the reagent in the Falck-Hillarp technique) to form fluorophores with biogenic indolamines and catecholamines Strong fluorescence is induced also from N-acetylated indolamines (e g melatonin) methoxylated catecholamines (e g 3-methoxytyramine) and peptides with tryptophan in NH_2- or COOH-terminal position; these compounds cannot be visualized with the standard Falck-Hillarp technique The fluorophores formed from primary and secondary phenylethylamines and indolamines in the GA reaction are strongly fluorescent isoquinoline and β-carboline derivatives

The GA method has been successfully applied to tissue where a precise and sensitive localization of intracellular monoamines both in neuronal and non -neuronal tissue is possible The new technique has proved extremely useful for sensitive and detailed neuroanatomical studies on catecholamine neurons in the CNS

Hökfelt T , Fuxe K , and Goldst in M (Department of Histology Karolinska Institutet 104 01 Stockholm Sweden and Department of Psychi try New York Medical Center New York U S A.): IMMUNOHISTOCHEMICAL VISUALIZATION OF CATECHOLAMINE SYNTHESIZING ENZYMES 60

Three catecholamine synthesi ing enzymes dopa deca boxylas (DDC) dopamine-β-hydroxyla (DBH) and phenyl thanolamine-N-methyltransf se (PNMT) we e purified from bovin adrenal med lla nd antibodi to th se enzymes (anti DDC anti-DBH and anti-PNMT) wer produ ed in rabbits Th antibodies were used in immunohistochemi al tudi (indirect techniqu) on the adrenal med lla and the pe ipheral and cent al nervous sy tem (CNS)

DDC and DBH wer present in all gland lls f the adrenal med lla whe eas PNMT could be demonstrated nly in a peci l population supporting the concept f two cell types in th ad enal medulla storing no ad enalin (NA) and adrenaline (A) respectiv ly Objectiv and subjective e timations f enzyme lev ls were perf rmed on the ad enals after various expe iment l proced res It was found e g that ACTH increased the PNMT lev l of hypophysectomized rats

In the CNS ll parts f the monoamin neu ons could be identified with thi t chniqu F rthermo dopamine (DA) (containing DDC but not DBH) and NA (ontaining DBH) neurons could be eparated 5-Hydroxytryptamin (5-HT) neurons could also be visuali ed with anti DDC suggesting that DA and 5-HT neurons ontain an ntigenically undi tinguishabl d carboxylas In addition t catecholamine sy tems described on the ba is f the Falck-Hillarp technique DDC-positive c ll ould be identified in c rtain hypothalamic areas

61 Ljungdahl, Å and Hökfelt T (Department of Histology Karolinska Institutet 104 01 Stockholm Sweden): AUTORADIOGRAPHIC DEMONSTRATION OF ^{3}H-GABA AND ^{3}H-GLYCINE UPTAKE IN SPINAL CORD

γ-aminobutyric acid (GABA) and glycine have been postulated to mediate pre- and postsynaptic inhibition in the spinal cord but the localization of the presumed GABA and glycine neurons is not known In the present study light and electron microscopical autoradiography has been used to locate the uptake of ^{3}H-GABA and ^{3}H-glycine in rat and cat spinal cord Both in vivo injections of the isotopes directly into the spinal cord and in vitro incubations of tissue slices were used

^{3}H-glycine is accumulated in nerve terminals often characterized by their content of flat vesicles in myelinated axons in glial cells and in certain neuronal cell bodies These cell bodies are mainly located in the anterior horn

^{3}H-GABA is accumulated in nerve terminals often making axo-axonic contacts with non-labeled boutons This is of interest in view of the proposed role of GABA in presynaptic inhibition ^{3}H-GABA is also accumulated in glial cell and neuronal cell bodies These neurons have a more dorsal location than ^{3}H-glycine accumulating cells

In contrast ^{3}H leucine is taken up by all cells with no accumulation in nerve terminals

Thus it is possible to map specific neuronal systems in the spinal cord by autoradiography with ^{3}H-GABA and ^{3}H-glycine

ERE!

62 Seiger, Å and Olson, L (Dept of Histology Karolinska Institutet Stockholm Sweden): HISTOCHEMICAL DEMONSTRATION AND MAPPING OF CENTRAL MONOAMINE NEURONS IN THE RAT: THE PRENATAL ONTOGENY

The development of all monoamine-containing neuron systems in the rat brain throughout the prenatal period of development has been analysed by Falck-Hillarp fluorescence histochemistry (Olson and Seiger Z Anat Entw Gesch 1972 301; Seiger and Olson Z Anat Entw -Gesch 1973 in press) It has been possible by serial sagittal horizontal and transverse sections through whole brains from 10 to 22 day old fetuses to map the development of 11 monoamine cell groups known in the adult animal a large number of axon bundles and their formation of terminal plexuses in different receptor areas Only fetuses from MAO-inhibited mothers were analyzed because of the marked increase of intraneuronal amine levels by this pretreatment

The first monoamine cell group to appear is the 5-HT group in rostral pons and caudal mesencephalon on the 12th day of gestation (crown rump length CRL 8 mm) The mesencephalic DA cell complex appears a little later (CRL 9 mm) and the pontic NA cell complex can be seen at the 14th day of gestation (CRL 11-12 mm) The common origin of different monoamine cell groups described in adult animal has been determined Thus A1-A3 A4 A7 A8 A10 B1-B3 B4-B9 respectively (nomenclature according to Dahlström and Fuxe 1964) have common origins New neuroanatomical data has been obtained mainly 5-HT axon projections not previously described Ascending bundles are seen from groups B6 B7 B8 B9 and probably from B3 and B5 Descending bundles are seen from groups B1 B2 B3 B6 and probably from B5 B7 B8 and B9 It is concluded that all the monoamine cell groups present in the adult animal appear and at least partly develop prenatally

DEVELOPMENTAL CHANGES OF THE NEUROTOXIC ACTION OF 6 HY DROXYDOPAMINE (6-OH DA) ON CENTRAL NORADRENALINE (NA) NEURONS Charlotte Sachs and Gösta Jonsson Dept. of Histology Karolinska Institutet Stockholm. 63

The neurotoxic substance 6-OH DA does not readily pass the blood brain barrier (BBB) However systemic injection of 6-OH DA to newborn rats produces a selective and permanent degeneration of a considerable number of NA nerve terminals in the brain (60% reduction of ^{3}H NA uptake in cerebral cortex and spinal cord; 40% reduction in the hypothalamus) thus indicating that 6-OH DA can pass BBB in the newborn stage In order to study the development of BBB to 6-OH DA rats received 6-OH DA (3x100 mg/kg s c.) at various periods of time after birth. The neurotoxic action of 6-OH DA was evaluated by measuring the in vit o uptake of ^{3}H NA in various brain regions when the rats had reached the adult stage There was a rapid development of a barrier and the ^{3}H NA uptake was completely restituted in the cereb al cortex when 6-OH DA was given on day 9 postnatally while in the hypothalamus on day 5 No effective barrier seemed to develop in the spinal cord. 6-OH DA per e had no appa ent effect on the outgrowth of the NA nerves that did not undergo degeneration. However analysis of ^{3}H NA uptak in ce bral cortex shortly after the 6 OH DA injection in rats older than 9 days showed a small (about 25%) t ansient reduction in ^{3}H NA uptake in rats younger than 28 days while in rats older than 28 days this effect e med permanent These results show that the BBB to the neurotoxic action of 6-OH DA although not completely protective develops shortly afte birth. The NA neurons possess up to about 28 days postnatally a certain regenerative capacity which late disappear

Olson, L. and Seiger, A (Dept f Hist logy Karolinska In tit t t Stockholm Sweden): BRAIN TISSUE TRANSPLANTED TO THE ANTERIOR CHAMBER OF THE EYE: MONOAMINE NEURONS REINNERVATING THE RAT IRIS 64

Transplant tions to the anteri y chamber has previously been used to study growth of sympatheti neurons (Olson and Malmfors Acta phy iol scand 1970 Suppl 348) The prenatal ontogeny of the CNS monoamine neurons has re ently been described (Olson nd Seiger Z Anat Entw Gesch 1972 137 301; Seiger and Olson Z Anat Entw Gesch 1973 in p es) Knowing the development of the noradrenaline (NA) d pamine (DA) and 5-hydroxytryptamin (5-HT) neurons it i po ible to obtain by mi rodi ection small pieces f immatu e brain containing th variou monoamine neurons F llowing homologou transplantation t ympathectomi ed y f ad lt rat th tr nsplants readily survive become v scularized and matur intraocularly The method permit in vivo bservations f the condition of the transplants

Th tr nsplant and i ids wer nalyzed by F lck-Hill rp fluor s enc histochemi try (Ol on and Seiger Z Zellf 1972 135 175) All three types f monoamine neu ons survived in the tr nsplant and innervated it in at least partly homotypical patt rns F rthermor the ntral monoamine neurons we all abl to reinnervat th symp thectomized host i is f rming plexu es imilar t the normally p nt sympath tic ground plexus Catecholamine nd 5-HT fiber ha ed the same pathway on the i is when tr nsplanted t g ther From thi and similarly designed experiment it i concluded that all the thr e type f mon amine neurons ar abl t einnervat denervated sympatheti rec ptor Th pattern f r innerv tion and morphology of the outgrowing fibe i d termined by th ecepto tissu

65 Tuomisto, J (Department of Pharmacology University of Helsinki Finland) DOPAMINE UPTAKE IN STRIATAL SYNAPTOSOMES CONFORMATIONAL SELECTIVITY

Noradrenaline (NA) and its sterically rigid analogs were used as inhibitors of dopamine (DA) uptake in striatal synaptosomes NA was rendered conformationally rigid by incorporating its active groups into the trans-decalin structure Four isomers were used in three of them the relation of the catechol ring and the amino function was gauche in one it was anti Crude synaptosomal fractions were prepared according to Snyder and Coyle (J Pharmacol exp Ther 165 78 1969) The synaptosomes were incubated with 10 7M tritiated DA and with or without an inhibitor for 5 min in Krebs Henseleit bicarbonate buffer One of the gauche isomers 2(a) amino 3(e) 3 4-dihydroxyphenyl 3 trans-decalol had 50% of the potency of NA as DA uptake inhibitor Its ID50 (concentration inhibiting 50% of uptake) was 2 4 x 10 6M The inhibition was competitive The anti isomer was 22 times weaker as an inhibitor (ID50 5 2 x 10^{-5}M)

These results suggest that the DA transport mechanism in striatal nerve terminals is conformationally selective and the conformer having the highest affinity for the transport is gauche It is noteworthy that according to molecular orbital calculations (Kier and Truitt J Pharmacol exp Ther 174 94 1970) the preferred conformation of DA is gauche but that of NA is anti Thus the transport mechanism in the striatum would favour DA for NA since only the former is predominantly in the gauche form

66 Squires, Richard (Research Laboratory A/S Ferrosan Soeborg Denmark) : EFFECTS OF SOME PSYCHOTROPIC DRUGS ON THE SIMULTANEOUS UPTAKE OF 5 HT AND NORADRENALINE BY SYNAPTOSOMES

Synaptosomes are prepared from rat forebrain by gentle homogenization in 0 32M sucrose containing 1mM Nialamide Unbroken cells and debris are removed by low speed centrifugation and the whole supernatant used as a source of synaptosomes Synaptosomes are mixed in ice bath with modified oxygenated Krebs Ringer test substance plus radioactive (C14) 5-HT (0 05µM) and (H3) noradrenaline (25µM) (final conc) The mixture is transfered from ice bath to 37° for 20 min then returned to ice bath to stop uptake Synaptosomes are then collected on Millipore filters washed with cold 0 9% NaCl and counted in Instagel in a liquid scintillation counter 5-HT uptake was inhibited 50% by ch orimipramine (14ng/ml) chlorpheniramine (94) imipramine (138) amitriptyline (168) protriptyline (566) and desmethylimipramine (623) The concentrations of these substances required to inhibit the uptake of NA 50% was generally much higher chlorpheniramine (0 22µg/ml) protriptyline (0 05)chlorimipramine (1 2) amitriptyline (1 4) desmethylimipramine(1 8) imipramine (2 0) A preliminary comparison of 5-HT NA and dopamine uptake inhibition in synaptosomes from different brain regions suggests that under the conditions described above 5 HT is taken up almost entirely by specific 5-HT synaptosomes while NA maybe taken up mainly by DA synaptosomes in all regions of the brain examined

Tuomisto L (Department of Pharmacology University of Hel inki Finland): INHIBITION OF SYNAPTOSOMAL UPTAKE BY TETRAHYDROHARMANE DERIVATIVES 67

Tetrahydroharmane alkaloids have an indolylethylamine moiety in their structure and it is feasible that these compounds may be formed in vivo as condensation products of 5-hydroxytryptamine (5HT) and acetaldehyde Effects of these compounds were studied on monoamine uptake in rat brain stem synaptosom s Crude synaptosomes were prepared according to Snyd r and Coyle (J Pharmacol exp Ther 1969 165 78) and incubated for 5 min with radioactive noradrenaline (NA) dopamine (DA) or 5HT in Krebs-Henseleit bicarbonate buffer 1 2 3 4-t trahydroharmane (TH) and 6 hydroxy-1 2 3 4-tetrahydroharmane (6HTH) were equipotent as catecholamine uptake inhibitor and the ID50 (con entration inhibiting 50% of uptake) was 2 5 x 10^{-5}M for NA and 7 x 10^{-5}M for DA As 5HT uptake inhibitor 6HTH was slightly more active (ID50 4 x 10^{-6}M) than TH (ID50 8 x 10^{-6}M) In all instance these compound were more potent than salsolinol which i the corre ponding cond nsation product of DA However also the p tency of TH and 6HTH was clearly lower than that of the parent amin s 5HT and tryptamine

It is concluded that tetrahydroharmane derivatives have affinity to the amine tran port mechanisms in the synapto ome but the affinity is lower than that of the primary indolylethylamines

Winbladh,B (Research Institute of National Defence Sundbyberg Sweden): CHOROID PLEXUS UPTAKE OF ACETYLCHOLINE: 68

Acetylcholine (ACh) is accumulated by rabbit choroid plexa in vitro by active transport The transport system seems to be identical with that for choline (Ch) and has a high capacity: Calculated maximal speed of uptake varied between 10 and 60 mmoles x min^{-1} x kg^{-1} wet weight The choroid plexa contained a significant cholinesterase activity (ChE) which seemed to be localized mainly intravascularly ACh was not metabolized by the tissue i e intravascular concentrations of ACh must have been low The cholinesterase inhibitor (ChEI) physostigmine inhibited ACh and Ch uptake whil the two ChEI:s soman and sarin stimulated the uptake The relative stimulation of the ACh uptake by soman increased with increasing ACh concentration in the incubation medium Soman enhanced the release of ACh from tissue preincubated for 15 min at 10^{-4}M but not from tissue preincubated at 10^{-7}M ACh in preliminary experiments The results will be discussed in relation to ACh and Ch transport in brain slices Further possible localizations of the transport mechanism will be suggested.

69 Nordberg,A and Sundvall,A (Dept of Pharmaceutical Pharmacology Biomedical Center Box 573 S-751 23 Uppsala Sweden):EFFECT OF PENTOBARBITAL ON THE TURNOVER OF ACETYLCHOLINE IN DISCRETE REGIONS OF MOUSE BRAIN IN VIVO

It has recently been shown that although barbiturate anaesthesia produces increased steady state levels of acetylcholine(ACh) in the brain it markedly decreases the turnover of the transmitter(Schuberth J Sparf B and Sundwall A J Neurochem 16 695(1969) Since cholinergic neurons are not evenly distributed it was considered of interest to study the effect of pentobarbital on steady state concentration and turnover in different regions of the mouse brain

The animals were killed by dislocation of the spine and the brains were rapidly dissected on ice into six welldefined parts(cerebellum medulla oblongata midbrain striatum hippocampus and cortex) The tissue was homogenized in TCA and the ACh in the extract was estimated by bioassay using a strip of the dorsal muscle of the leech in a microbath The results are summarized in the table As seen there were marked differences regarding regional ACh values both in normal and pentobarbital treated animals

ACh in nanomoles/g brain

	Cerebellum	Medulla obl	Midbrain	Striatum+	Hippocampus	Cortex
Control	5 73	22 77	18 11	36 85	17 40	11 14
%increase	n s	n s	57	n s	79	163

+ 2/5 samples 125% increase

Turnover was studied by measuring the rate of formation of radioactive ACh in different parts of the brain following intravenous injection of radioactive choline(Ch) A marked decrease was demonstrated in hippocampus and cortex following an anaesthetic dose of pentobarbital

70 Gudrun Paalzow and Lennart Paalzow (Departments of Pharmacology, B M C , Box 573 S-751 23 Uppsala and Apotekens Centrallab , Box 3045, S-171 03 Solna 3 Sweden): THEOPHYLLINE INCREASED SENSITIVITY TO ... STIMULATION AND REGIONAL TURNOVER OF RAT BRAIN 5-HT ... HIAA NORADRENALINE AND DOPAMINE

We have previously reported (Acta pharmacol et toxicol 32,22, 1973) that theophylline and caffeine are able to decrease the normal pain threshold in a dose-dependent manner These methylxanthines are known to increase the level of cyclic AMP and considerable evidence suggests that cyclic AMP may participate in the synaptic transmission We have now looked more closely upon the relationship between the increased sensitivity to painful stimulation after administration of theophylline and the regional turnover of rat brain indole- and catecholamines

The rats were killed 30 min. after injection of 100 mg/kg theophylline i p and the brain were dissected into three parts; cortex, forebrain and brain stem. Responses to nociceptive stimulation were divided into motor response vocalisation and vocalisation afterdischarge

Theophylline was found to be able to decrease the turnover of dopamine in cortical and forebrain regions while that of noradrenaline was unaffected An increased level of 5-HIAA was found in the brain stem following theophylline and this increase was more pronounced after probenecid treatment In cortical regions the level of 5-HIAA was decreased Endogeneous serotonin were unaffected in all regions studied Probenecid per ___ antagonized the theophylline decreased threshold for vocalisation afterdischarge and the cortical decreased 5-HIAA

Wiesel, F A , Fri, C G and Sedvall, G (Dept of Pharmacology Karolinska Institutet Stockholm Sweden) EFFECT OF PARGYLINE ON HOMOVANILLIC ACID IN RAT STRIATUM AS MEASURED BY MASS FRAGMENTOGRAPHY 71

Earlier methods have lacked enough sensitivity for determination of homovanillic acid (HVA) turnover in rat striatum using a monoaminoxidase inhibitor This has now been possible since a mass fragmentographic method has been developed for measuring HVA in the picomole range in brain tissue (Sjöquist et al J Neurochem 1973 in press)

Male Sprague Dawley rats were injected with pargyline 75 mg per kg i v and killed at different time intervals Striata were homogenized in 0 1 M formic acid and 50 μM ascorbic acid Each sample was divided for determination of HVA and dopamine (DA) (Koslow et al Science 1972 176 177) The steady state levels of HVA and DA were found to be 4 and 57 nmole/g respectively After pargyline treatment the HVA levels disappeared following an exponential curve The half life was calculated to 12 minutes HVA turnover was estimated to be at least 14 nmole per g per h This is in agreement with data found by Spano and Neff (1972) using probenecid as a tool for calculating HVA turnover in guinea pig striatum The DA levels increased about 40% within 80 minutes

Supported by the Swedish Medical Research Council (04X 2381) NIMH Bethesda USA (MH15755 04) and Magnus Bergwalls stiftelse

Lindqvist M, Carlsson A, and Kehr W (Department of Pharmacology University of Göteborg Sweden): INFLUENCE OF ANAESTHETICS ON TYROSINE AND TRYPTOPHAN HYDROXYLATION IN RAT BRAIN IN VIVO 72

Rats were exposed to ether during 15–60 min and NSD 1015 (3-hydroxybenzyl hydrazine HCl) an inhibitor of aromatic amino acid decarboxylase 100 mg/kg i p was given either during or at arious times after ether anaesthesia Ether caused a rapid acceleration of dopa and 5-HTP accumulation after decarboxylase inhibition during the initial 15–30 min of anaesthesia while the accumulation was decreased below control values in the period after ether These changes in tyrosine hydroxylation were most pronounced in the limbic DA- ich system and especially in the corpus striatum whereas the changes in tryptophan hydroxylation were less marked and about similar in all brain parts

Similar changes in tyrosine hydroxylation were also observed after ethanol treatment (0 5–4 g/kg) After NSD 1015 the dopa accumulation in the brain was accelerated by ethanol both in the corpus striatum and in the hemispheres However no concomitant effect on 5-HTP levels was found

Nembutol anaesthesia (40 mg/kg) had no marked influence on monoamine synthesis in the brain

Ether and ethanol seem to have similar actions on monoamine synthesis in catecholamine neurons but only ether has in addition an effect on 5-HT neurons

73 Schubert J Fyrö B and Sedvall G (Dept of Psychiatry (St Göran s Hospital) and Pharmacology Karolinska Institutet Stockholm Sweden) SYNTHESIS OF MONOAMINES FORMED FROM LABELLED PRECURSORS IN RAT BRAIN DURING LITHIUM TREATMENT

Chronic treatments with lithium were performed with two doses of LiCl added to the food resulting in lithium levels in serum of 0 4 and 0 7 meq/l respectively At the lower dose monoamine synthesis in brain was not affected With the higher dose accumulation of labelled 5 hydroxytryptamine (5 HT) and 5 hydroxyindoleacetic acid in brain during i v infusion of ^{3}H tryptophan was increased This effect was present in the spinal cord following acute spinalization thus occurring independently of nerve impulse activity Disappearance from brain of labelled 5-HT formed from ^{3}H tryptophan was reduced in lithium treated animals indicating that lithium diminishes release of 5 HT from its storage sites Brain tryptophan was increased after lithium whereas levels of free and total tryptophan in serum as well as its catabolism reflected by liver tryptophan pyrrolase activity were unaltered Accumulation and disappearance of labelled dopamine and noradrenaline formed from ^{14}C tyrosine in brain were not changed significantly by any of the lithium diets

The results suggest that chronic treatment of rats with lithium within the low dose range recommended for therapy in man affects 5 HT but not catecholamine synthesis in brain

Supported by grants from the Swedish Medical Research Council (14X 2381 and 21X 2291) and Karolinska Institutet

74 Amtorp O & S C Sørensen (Institute of Medical Physiology Dept A University of Copenhagen Denmark): ONTOGENESIS OF THE -BRAIN BARRIER IN RATS:

The exchange of hydrophilic substances between blood and interstitial fluid is impeded in the brain compared to other tissues Furthermore the socalled blood-brain barrier is able to maintain concentration differences for various ions by means of active transport mechanisms We have in rats studied the development of the barrier to macromolecules and of the transport processes for K^+, Na^+ and Cl^- by measuring the concentration of total protein K^+ Na^+ and Cl^- in cisternal cerebrospinal fluid (csf) and plasma from newborn rats and from rats of various ages The concentration ratio csf/plasma (mean±SD) for proteins was 0 091±0 007 at birth and did not reach the adult value of 0 0053 0 0001 until 24 days after birth In contrast the concentration ratios csf/plasma H_2O for K^+ Na^+ and Cl^- were at the time of birth not significally different from the values found in adult rats i e 0 63±0 07 0 99±0 02 and 1 15±0 02 respectively Thus the ability to maintain concentration differences for small ions develops before the blood-brain barrier becomes tight to macromolecules This may indicate that the tightness to macromolecules and the active transport of small ions may be attributed to different cell layers e g endothelium and perivascular astrocytes respectively It must however also indicate a large capacity of transport systems of the blood-brain barrier enabling maintenance of concentration differences despite a large intercellular leak

Lund-Andersen H & M Møller (Department of Biochemistry A and Anatomy Department A University of Copenhagen Denmark): EXTRACELLULAR AND INTRACELLULAR DISTRIBUTION OF INULIN IN RAT BRAIN-CORTEX SLICES 75

Inulin space is commonly used to indicate the magnitude of the extracellular space in brain-cortex slices It has however been shown (Lund-Andersen & Hertz Biochem Soc Trans 1 1973 123) by examination of the wash out course of ^{14}C-labelled inulin that 2/3 of the amount taken up during 60 min of incubation is localized in the extracellular space whereas 1/3 is intracellularly located The 'true extracellular space can be calculated to 29 µl per 100 mg initial wet weight A large extracellular space compared with unincubated slices is correspondingly observed in electron micrographs The amount of extracellularly located inulin is reduced by 50 per cent when cellular swelling is evoked by 35 mM KCl; electron micrographs confirm the reduction of the extracellular space and furthermore shows that the KCl evoked swelling is a glia cell phenomenon (Zadunaisky et al Exp Neurol 8 1963 290)

It is concluded 1) that the quantitative determination of the magnitude of the intra- and extracellular compartments by aid of inulin diffusion kinetics is in agreement with the qualitative electron microscopically findings 2) That the potassium-induced swelling is a glia cell phenomenon 3) That intracellular penetration of inulin in brain tissue may be a physiological phenomenon or may indicate in vitro cell membrane damage 4) and that the traditionally defined inulin space is not a measure of the extracellular space in brain-cortex slices

Aarseth P and B A Waaler (Institute of Physiology University of Oslo Norway): INTERSTITIAL WATER CONTENT IN ISOLATED RABBIT LUNGS AT DIFFERENT LEVELS OF CAPILLARY PRESSURE 76

It has been questioned whether the Starling concept for transcapillary fluid balance applies to the pulmonary vascular bed The existence of a small volume of interstitial fluid with a large protein-concentration could explain how changes in hydrostatic capillary pressure is balanced without much extra accumulation of perivascular fluid We wanted to test whether there is only such a small augmentation of extravascular fluid after elevation of lung capillary pressure Isolated rabbit lungs were perfused with plasma and their weight continuously followed Extravascular water content and plasma volume were calculated in removed lobes with the use of labelled plasma albumin and by measurement of wet weight/dry weight ratios At stable vascular pressures the extravascular water content of the upper lobes differed by less than 3% In test experiments the left atrial pressure was elevated by 10 mm Hg One upper lobe was removed before the other 8 min after this pressure elevation At this time the preparations were approaching weight stability indicating that transcapillary fluid balance was nearly achieved In the lobes exposed to such elevated vascular pressures we could either record no changes in extravascular water content or an augmentation of 10% or less on y The extravascular fluid compartment involved in transcapillary fluid balance in the lungs is thus apparently very small

77 Parving H -H, Rossing N Nielsen S E & Lassen N A (Department of Clinical Physiology Bispebjerg Hospital Copenhagen Denmark): INCREASED TRANSCAPILLARY ESCAPE RATE OF PLASMA PROTEINS DURING ALBUMIN AND DEXTRAN LOADING IN MAN

The transcapillary escape rate (TER) of a plasma protein is defined as that fraction of the intravascular mass of the protein which passes to the extravascular space per unit time TER of plasma albumin was determined as the initial disappearance rate of i v injected radioiodinated albumin in 4 human subjects before and after i v loading with 95 g albumin Albumin loading significantly increased TER from 4 6 (S D ±0 8) to 8 1 (S D ±2 0) %/hr The consequence was a net transfer of 11% of the plasma albumin mass to the extravascular space during the first 5 hrs after the loading There was also a net transfer of other proteins as reflected by a reduction of the masses of intravascular IgG and IgM of 9 and 7 5% respectively Similar results were obtained in 4 other subjects loaded with 900 ml Dextran (Mw 70 000)

Our results can be explained by increased microvascular permeability (the stretched pore phenomenon) and/or by increased filtration due to the venous pressure elevation caused by the loading

78 Poulsen, H L (Department of Clinical Physiology Bispebjerg Hospital Copenhagen Denmark): SUBCUTANEOUS INTERSTITIAL ALBUMIN CONCENTRATION IN LONG-TERM DIABETES MELLITUS

By a wick method human interstitial fluid was collected the subcutaneous tissue of the forearm 12 normal men had an average interstitial fluid albumin concentration of 2 66 g/100 ml (S D 0 30) and a ratio between interstitial albumin concentration and serum albumin concentration of 0 63 (S D 0 09) 8 male juvenile long-term diabetics matched for age, had the corresponding values of 1 46 g/100 ml (S D 0 47) and 0 38 (S D 0 12) respectively (p<0 001) In 3 male juvenile short-term diabetics normal values were found

In view of other reports pointing to increased microvascular albumin permeability in long-term diabetes the results suggest a much enhanced net water flux (lymph formation) in the tissue studied

Fadnes, H.O. (Institute of Physiology University of Bergen Norway): INTERSTITIAL FLUID PRESSURE AND ALBUMIN CONCENTRATION IN EXPERIMENTAL HYPOPROTEINEMIA 79

Absence of edema in hypoalbuminemia suggests compensatory changes in the transcapillary Starling forces for instance 1) increased interstitial hydrostatic pressure (P_i) or 2) a greater reduction of albumin concentration in interstitial fluid (A_i) than in plasma (A_p)

These possibilities were investigated in rats during development of aminonucleoside nephrosis P_i was measured with a modified Scholander wick method with the wick contained in a 20 gauge cannula provided with a sidehole A_i was determined in wicks after 1 hour subcutaneous implantation (Aukland & Fadnes Acta physiol scand 1972 84 26A) Daily injections of 5-6 mg Puromycin aminonucleoside induced proteinuria and progressive hypoalbuminemia after 4-5 days P_i ranged from 0 to -2 cm H_2O both under control conditions and in hypoalbuminemia Control A_p and A_i averaged 3 5 and 2 0 g/100 ml At the 5th day A_p was reduced by about 1 g/100 ml and an even greater reduction was observed in A_i when A_p was further reduced to less than 2 g/100 ml A_i fell to less than 0 3 g/100 ml

It is suggested that the disproportional reduction of A_i is caused by increased capillary filtration and lymph flow and in turn acts to limit edema formation P_i does not seem to be of importance for preventing subcutaneous edema

Auranes I and J Vaage (Institute of Physiology University of Oslo Norway) CHANGES IN PERIPHERAL LYMPH DURING INTRAVASCULAR AGGREGATION OF BLOOD PLATELETS 80

Several investigators claim that blood platelets may yield components affecting vascular permeability after various types of in vitro treatment We wanted to study the effects on microvessels of intravascular aggregation of blood platelets

The number of erythrocytes was estimated in peripheral lymph collected from a major lymph vessel in the hind limb of cats Blood pressure (BP) hematocrit (hct) and the number of circulating blood platelets were followed Intravascular platelet aggregation was induced by infusion into the abdominal aorta over one hour of a collagen suspension

During this infusion the BP decreased moderately The level of circulating blood platelets fell to about 30 per cent of the initial value The number of erythrocytes in the lymph normally less than 1000 per mm³ increased to a peak value of about 10000 per mm³ Hct rose by about 25 per cent and lymph flow increased considerably

When the collagen infusion was ended hct and lymph flow rapidly decreased The number of erythrocytes in the lymph did gradually return to a normal value during the next 3 hours

We conclude that intravascular aggregation of blood platelets can induce acute changes in the wall characteristics of the microvessels This change may be mediated through substances released from the blood platelets

81 Paaske W P & B Levin Nielsen (Institute of Medical Physiology B University of Copenhagen Denmark & Department of Clinical Physiology Bispebjerg Hospital Copenhagen Denmark): VASCULAR PERMEABILITY IN ADIPOSE TISSUE MEASURED BY THE SINGLE INJECTION EXTERNAL REGISTRATION METHOD

Measurement of capillary permeability in adipose tissue with available methods has proved difficult due to the low perfusion of this tissue in vivo The single injection external registration method using bolus injection of gamma-emitting radionucleides (Sejrsen P In Capillary Permeability Munksgaard, Copenhagen 1970) was therefore applied to adipose tissue

The present study describes the theoretical basis of the method and suggests a technique of fast on-line computation of capillary extraction and capillary diffusion capacity combined with graphical plotting of the externally recorded triexponentially fitted curve function

After bolus injection of ^{51}Cr-EDTA into the artery of the isolated inguinal fat pad in slightly fasting female rabbits the capillary extraction and capillary diffusion capacity were found to be essentially similar to values reported from experiments on skeletal muscle It is concluded that the single injection external registration method is of considerable value for determination of capillary permeability in adipose tissue

82 Aarseth,P ,G Bø and H Piene (Institute of Physiology University of Oslo):PLASMA HYPEROSMOLARITY AND PULMONARY FLUID COMPARTMENTS

Plasma hyperosmolarity has a marked dilatory effect on muscle istance vessels and some dilatory effect also on pulmonary tance vessels In contrast to this systemic capacitance vessels are not much influenced by hyperosmolarity The pulmonary capacitance vessels of isolated lungs appeared to constrict when the plasma osmolarity was raised (Bø Hauge and Nicolaysen Acta physiol scand 1971 82 375).

In the present investigation we have estimated the blood- and extravascular water volumes in the lungs of 7 intact cats during infusions of hyperosmotic solutions (3M NaCl 3M and 5 9 M glucose) These volumes were estimated by measuring plasma and erythrocyte radioactivity in rapidly snared-off lung lobes After a mean increase of 18% in the plasma osmolarity the blood content of a lobe No 2 was increased by 24% relative to that of a lobe No 1 removed before infusion This increase is however not significantly greater than what was found in a control group of 5 cats where the 2 lobes were removed without any intervening infusions The extravascular water volume during hyperosmolarity fell by 21% which is significantly different from a minor change seen in the control group

It is concluded that increase in plasma osmolarity has no detectable effect on the pulmonary capacitance vessels whereas it will markedly reduce the extravascular lung water volume

Nilsson, G. (Department of Pharmacology, Karolinska Institute, Stockholm, Sweden): EFFECT OF ACID IN THE DUODENAL BULB ON FASTING GASTRIC AND GASTRIN SECRETION 83

Bulbar acidification inhibits gastric acid secretion in response to test meal sham feeding, insulin hypoglcemia, exogenous gastrin but not to histamine The present study was undertaken to determine if bulbar acidification inhibits fasting gastric acid secretion and basal plasma levels of gastrin

Dogs were provided with fully innervated pouches of the fundic stomach and of the proximal portion of the duodenum corresponding the duodenal bulb Acid perfusion of the bulbar pouches abolished the spontaneous acid secretion from the Pavlov pouches but did not influence the plasma concentration of gastrin

The results may mean that lowering of the intrabulbar pH plays a role in suppressing gastric acid secretion appearing between meals Such inhibition is not induced by reduction of plasma concentrations of gastrin Instead it may be due to the hypothetical hormone bulbogastrone acting at the acid secreting glands

Sjödin L, and Nilsson, G (Department of Pharmacology, Karolinska Institutet, Stockholm, Sweden): GASTRIC ACID SECRETION AND PLASMA GASTRIN LEVELS DURING URECHOLINE INFUSION IN THE DOG 84

Cholinergic stimulation of gastric acid secretion may be induced by different means such as sham feeding or hypoglycemic agents Such cholinergic activation of acid secretion is thought to be due to both direct excitation of the parietal cells and release of antral gastrin. In the present study, the effects of a stable choline ester (Urecholine[R]) on gastric acid secretion and plasma gastrin levels were investigated

Each of 4 dogs was provided with a vagally denervated fundic pouch and a draining cannula in the main stomach A continuous intravenous infusion of 50 µg per kg-hr of Urecholine did not influence plasma gastrin concentration as determined by radioimmunoassay Administration of 100 µg per kg-hr significantly raised the plasma gastrin level in only 3 out of 10 experiments However, in all experiments, Urecholine did cause marked acid secretion from the gastric pouches

The results show that cholinergic stimulation by Urecholine may evoke gastric acid secretion without influencing the plasma level of gastrin This suggests that basal plasma levels of gastrin suffice to potentiate the direct effect of Urecholine on the parietal cells

85 Berglindh T K,J Öbrink and M Waller (Institute of Physiology and Medical Biophysics University of Uppsala Sweden) VASOPRESSIN AS INHIBITOR OF ACID SECRETION IN THE ISOLATED GASTRIC MUCOSA

Isolated gastric mucosae from Rana Temporaria were mounted as a membrane in a two chamber system The nutrient side chamber contained a buffered frog-Ringer s solution gassed with 5% CO_2 and 95% O_2 The secretory side faced an unbuffered salt solution gassed with pure O_2

The mucosae were not stimulated unless they showed a complete secretory rest Then histamine to a final concentration of 10^{-7} mol/lit was given Consequently there were two sets of preparations: 1) those with a spontaneous secretion and 2) those with a histamine induced secretion

Lysin-vasopressin was added to the nutrient side to a final concentration of approximately 10^{-8} mol/lit The effect was a striking inhibition of the spontaneously secreting stomachs but no effect at all of the histamine stimulated ones

86 Frenning, B (Institute of Physiology and Medical Biophysics University of Uppsala Sweden) NET TRANSPORT OF SODIUM IONS INTO THE RESTING STOMACH

mM HCl was instilled into resting cat stomachs mounted in vivo as the of a cylindrical perspex chamber (Frenning B Upsala J Med Sci 1972 Suppl.13) A net movement of sodium ions into the instillate without any change of its volume was observed This result suggests a diffusional transport of sodium ions into the stomach (Teorell,T J gen Physiol 1939 23 263) However other authors believe that sodium ions enter the stomach merely as constituent of a diluting and/or neutralizing secretion (for review see Hunt J N and Wan B Handbook of Physiology sect 6,II p 781,1967)

To examine further the net movement of sodium ions into the resting stomach 170 mM HCl was instilled into the chamber and physiological saline or 170 mM HCl infused into the instillate at different constant rates At the end of the experiments the total amount of sodium ions in the gastric contents was determined and the amount infused subtracted When physiological saline was infused it was found that the amount of sodium ions that moved into the stomach (corrected for that infused) was lower the higher the infusion rate When 170 mM HCl was infused the net movement of sodium ions into the stomach remained at the control level

The results suggest that a considerable part of the net transport of sodium ions into the stomach occurred by diffusion

Rasmussen S (Department of Pharmacology Royal Danish School of Pharmacy DK-21oo Copenhagen Ø Denmark): THE EFFECT OF PHOSPHATE ON CALCIUM TRANSPORT IN ISOLATED RAT SMALL INTESTINE IN VITRO 87

Unidirectional Ca-fluxes were measured in isolated rat distal jejunum in a modified Ussing-apparatus At Ca-concentration of o 25 mM the following fluxes as nMol/cm^{2}h±s e m were measured:

mM PO_4	J_{ms}	J_{sm}	J_{net}	
0	5 9±o 2 (5)$^{x)}$	5 1±o 3 (5)	o 8	P>o 1
1	7 5±o 2 (6)	4 9±o 2 (6)	2 6	P<o oo1
2 5	7 o±o 4 (5)	4 6±o 5 (5)	2 4	P<o o1
5	6 4±o 2 (5)	4 4±o 2 (5)	2 o	P<o o1

J_{ms}: flux mucosa to serosa J_{sm}: flux serosa to mucosa J_{net}: netflux x): No of experiments The results are in agreement with those by Helbock et al (Biochim Biophys Acta 126 1966 81) who found net Ca-transport only in the presence of phosphate but in contrast to other authors e g Wasserman & Taylor (Mineral Metabolism Vol III C L Comar & F Bronner Ed Academi Press 1969 p 321) who did not find phosphate necessary for the net-transport

Damgaard Nielsen G & S Rasmussen (Department of Pharmacology Royal Danish School of Pharmacy DK-21oo Copenhagen Ø Denmark): EFFECT OF VITAMIN D ON THE CALCIUM INFLUX ACROSS THE BRUSH BORDER MEMBRANE OF ISOLATED RAT SMALL INTESTINE IN VITRO 88

Influx of Ca at 2o mM across the brush border membrane of the rat distal jejunum was measured by the method by Schultz et al (J Gen Physiol 5o 1967 1241) modified for rat intestines Influxes measured in rats bred and maintained on a vitamin-D free diet (all kindly supplied by Statens Levnedsmiddelinstitut Copenhagen) were found to be 2 12± o 11 µMol/cm^{2}h No of experiments: 7 Vitamin-D deficient rats kept on a rachitogenic diet for three weeks show influxes of 1 86±o 15 (7) µMol/cm^{2}h After administration of cholcalciferol 15oo i e and 3ooo i e orally to deficient and rachitic rats respectively the fluxes were found to be 2 6±o 3 (19) and 1 8±o 3 (8) µMol/cm^{2}h Treatment of vitamin D deficient rats with 3o i e daily did not change the Ca-influx The influx across the brush border membrane of Ca in the distal jejunum of rats kept on vitamin-D free diet is now influenced by vitamin-D administration

89 Olsen I and E. Sögnen (Department of Pharmacology and Toxicology Veterinary College of Norway Oslo Norway): INTESTINAL GLUCOSE TRANSPORT IN VITRO INFLUENCED BY CHLORHEXIDINE AND LAURYLSULPHATE.

Recently it has been shown that considerable amounts of chlorhexidine and laurylsulphate from mouth rinses will be retained in the oral cavity and swallowed thereby exposing the gastrointestinal tract The purpose of this study was to compare the effects of these drugs on an essential digestive process glucose absorption

Sacs of rat everted small intestine were filled with and suspended in glucose-containing Krebs-Ringer bicarbonate saline and incubated for 1 h at 37° C in the presence of chlorhexidine acetate or sodium laurylsulphate Histological sections from the loops were made

Except for 0 05 mM laurylsulphate which had a stimulating effect glucose transport was decreased progressively within the range of 0 01-1 00 mM chlorhexidine or laurylsulphate On an equimolar basis chlorhexidine was the most potent inhibitor A 50 % inhibition occurred at approximately 0 08 mM chlorhexidine and 0 40 mM laurylsulphate respectively The extent of mucosal disintegration could be related to the degree of drug inhibitory effect

It is concluded that chlorhexidine in oral therapeutics is unlikely to affect glucose absorption essentially more than the commonly used laurylsulphate since higher concentrations of the latter are employed

90 Tindall, A.R (Institute of Biology and Geology University of Tromsø Norway) Gastroliths in Norwegian grouse (Lagopus lagopus)

During the summer the stones in the gizzards of wild Norwegian grouse are smaller and more numerous than in winter In late winter a few grouse have been found without stones in their gizzards Captive grouse kept during the winter on standard grit are found to have stones typical of the summer-time throughout the year This shows that the composition is determined by the availability of stones rather than by an internal selection mechanism. In a few grouse however which have died in captivity the gizzard was completely blocked with stones

Experimental work has shown that when a domestic fowl is force-fed with glass beads some beads are excreted within the first few days others being retained for months Gravity seems to play a part in the retention of stones in the gizzard

Calcareous stones dissappear within a few days presumably dissolved by the acidity of the stomach although stones shaken with a solution of HCl were not readily dissolved

Ekelund M , K Lundegård and F J Öbrink (Institute of Physiology and Medical Biophysics University of Uppsala Sweden) THE ISOLATED MAMMALIAN GASTRIC MUCOSA IN HIGH OXYGEN PRESSURE 91

Contrary to isolated gastric mucosae from frogs those of mammals become hypoxic when gassed with oxygen at atmospheric pressure A pressure of 3200 mm Hg is necessary for a complete oxygenation

A chamber has been constructed in which such a high oxygen tension can be maintained and still the usual procedures for the experiment can be carried out such as gas-bubbling PD measurement electrical shortcircuiting continuous titration of the acid secretion with a pH-stat method and administration of drugs

Isolated mucosae of rats have been used and found to function with a high secretory rate in this oxygen pressure system The method is currently being used for a study of the histamine pentagastrin cyclic AMP and phosphodiesterase inhibitors on the gastric secretion

Appelberg,B (Dept of Physiology Faculty of Medicine University of Umeå 901 87 Umeå Sweden); Skel to- and fusimotor activation in the flexion reflex. 92

In dec rebrate and unanesthetized cats spinalized at low thoracic level the activity in primary muscl spindle afferents from the peroneus tertius or the emitendinosus muscle was recorded in dorsal root filament Other muscles in th hind limb were denervated. The cutaneous component of th superficial peroneal nerv was left intact and so were the dorsal and ventral roots except *for the dorsal root filament recorded from* In such preparations a reflex contraction of the muscle under study could be evoked by strongly pinching the paw In most preparations the spindles were not unloaded by the contracti n but instead their discharge was retained or even accelerated This indicates a simultaneous outflow of skeletomotor and static fusimotor activity in th fl xion reflex Attempts were also made t test the dynamic s nsitivity of the spindle by applying linear extension to the mus le before and during reflex contractions It was con sistently found that the dynamic burst of activity was not increased during the reflex Essentially similar findings were obtained in preparations where extrafusal but not intrafusal contractions were eliminated by critical curarisation It is concluded that dynamic fusimotor activity is not significantly increased during the flexion reflex

Dyhre-Poulsen P & Hounsgaard J (Laboratory of Behavioral Physiology Institute of Neurophysiology University of Copen hagen Denmark): TRANSMISSION OF AFFERENT IMPULSES THROUGH THE LEMNISCAL SYSTEM DURING A CONDITIONED MOVEMENT IN THE MONKEY 93

Previously it has been shown that the amplitude of potentials in the medial lemniscus evoked by electrical stimuli applied to a cutaneous nerve in the cat is reduced during and before a movement of the limb To determine whether a similar inhibition exists in primates as well monkeys were trained to depress a lever partially maintaine a fixed posture and depress the lever fully at the onset of a tone Electrical stimuli were applied to a periferal nerve before and during the conditioned movement and potentials were recorded between a gross electrode in the medial lemniscus and an indifferent electrode

The amplitude of the potential from the medial lemniscus was depressed during and before movements beginning around 2oo ms before movement of the limb As in the cat the decrease varies with the velocity of the limb Furthermore the decrease varies inversely with the stimulus intensity Stimuli just above threshold at rest evoke potentials that are more reduced during movement than potential evoked by stronger stimuli This may be interpreted to indicate that the pool of inhibitory interneurons is constant or has a maximum and that the inhibition includes transmission through large possibly muscular afferents

Johans on H & Silfvenius H (Dept of Physiology University of Umeå, Sweden): DO THE DORSAL SPINOCEREBELLAR- AND THE HIND LIMB GROUP I CEREBRAL TRACTS HAVE CO..ON SPINAL AXONS 94

It has recently been shown that spinal axons of the hind limb roup I pathway proj cting to the contralateral cerebral cortex ascend together with the axons of the dorsal spinocerebellar tract, D C^(Landgren S & Silfvenius H J Physiol 1971 218 551) It is not known, however whether or not these two ascending tracts have common spinal axons The problem is investigated in the present study using unitary recording from electrically activated bulbo thalamic relay cells in the region of the medullary nucleus Z

Three groups of neurones were observed: a) cells uninfluenced by lectrical surface stimulation of the anterior cerebellar lobe ipsi lateral to the spinal axons b) cells activated at short latency from the anterior cerebellar lobe but not by collaterals of the D C and c) c lls activated at short latency from the anterior r b llar lob via collaterals of the D.CT

It is concluded that the existence of relay cells in nucleus Z of typ s a and b favours the interpretation that the spinal component of th hind limb group I pathway to the contralateral cerebral cortex is not identical with that of the DSCT

Mak, P Klootwijk R Miller S, and van der Burg J, (Department of Anatomy, Erasmus University Rotterdam, Rotterdam, The Netherlands): STEPPING PATTERNS OF NORMAL AND DECEREBRATE CATS. 95

In normal cats trained to step on a treadmill all forms of stepping can be described by two patterns: 1 ALTERNATE STEPPING, where hindlimbs step alternately as in walking and trotting and 2 IN-PHASE STEPPING, where hindlimbs step mainly together, as in galloping (Miller et al , J Physiol (Lond.) 1973 230 30P) In both patterns a constant time coupling exists between the first extension of the hindlimb and flexion of the ipsilateral forelimb Similar stepping patterns with similar coupling of limb movements can be induced by electrical stimulation of the midbrain in decerebrate cats stepping on a treadmill (technique of Shik et al Biofyzika 1966 11 659) The coupling of hind- and forelimb movements is not abolished by bilateral section of dorsal roots between L_2 and S_2

From these results and from neurophysiological investigations of long propriospinal paths (Miller et al Brain Res. 1973 In Press) it is suggested that the pattern of stepping and the co-ordination of the four limbs are mainly organized in the spinal cord and are not primarily dependent on hindlimb afferent input

Jansakoq,T (Dept of Phy iology Univ of Umeå Swed n): CONVERGENCE FROM THE MESENCEPHALON AND THE SPINAL CORD IN CLIMBING FIBRE PROJECTIONS TO THE PARAMEDIAN LOBULE 96

Miller Nealina and Oscarsson (Brain Res 1969 14 234) showed that electrical stimulation within two restricted areas of the mesencephalon (Mes) evokes climbing fibre (CF) activity in the cerebellar anterior lobe of the cat with the olivary neurones involved sharing also the hind limb component of the dorsolateral spino-olivocerebellar path (DLF-SOCP)

The present investigation in halothane-anesthetized cat showed that electrical stimulation in the region of the dorsal part of the red nucleus (dorsal area of those mentioned abov) evokes CF activity in three di tinct parts of the paramedian lobule (PM) namely caudally in the lateral (c-l) and medial (c-m) thirds and rostrally in the medial (r m) third It was demonstrated that the c l projection shows convergen e from Mes and the hind limb DLF-SOCP Furthermore then activating DF-SOCP (dorsal funiculus) in C2 (both hind and for limb components) a convergence from Me to c-m PM and r m PM respectively could be shown This would be expected becaus the DF-SOCP by CF branching is known to project both to the anterior lobe and to the c-m (hind limb) and r-m (fore limb) PM

One explanation for thi more extensiv projection from Mes to the PM is that in addition to branching CF there might be CFs destined solely for the PM. This would result in a PM information partly different from that to th anterior lobe

Lukkari, J (Institute of Physiology University of Helsinki 97
Finland): DEVELOPMENT OF CEREBRO-CEREBELLAR AND CUTANEO-CEREBELLAR RESPONSES IN GUINEA PIGS

The development of cerebellar responses to electrical stimulation of the perioral skin and cerebral cortex was studied in adult and new-born guinea pigs and intra-uterine fetuses Cerebellar responses to ten electrical stimuli were recorded in the midline cortex of Larsell s lobules V-VI and averaged with a µ-Linc computer

In adults and new-borns the responses to peripheral stimuli consisted of five deflections the first of which was positive. In full-term fetuses only three and in 50-days fetuses only two such deflections could be seen The latency of the first positive peak was 10 msec in adults and new-borns, 30 msec in full-term and 45 msec in 50-days fetuses

The cerebellar responses to stimuli of primary sensory cortex were of the same shape as the responses to cutaneous stimulation In adults and new-borns the latencies of the first positive peaks were 5-8 msec in full-term fetuses they were 30 msec and in 50-days fetuses 60 msec

From these studies it seems that the fast climbing fiber responses in guinea pig cerebellum develop at the time of birth, though the mossy fiber responses are present much earlier

Secher N , Ruberg Larsen, N Binkhorst R A and Bonde 98
Petersen, F (Laboratory for the Theory of Gymnastics University of Copenhagen Denmark) MAXIMUM AEROBIC POWER DURING COMBINED ARM PLUS LEG EXERCISE RELATED TO THE STATE OF ARM TRAINING

16 subjects (11 arm and two leg-trained athletes plus three non athletes) were measured for maximum oxygen uptake rate ($\dot{V}_{O_2}$ max) and blood lactic acid during arm work leg work and arm-plus leg work $\dot{V}_{O_2}$ max was in addition measured for some of the subjects during treadmill running and bicycling Arm $\dot{V}_{O_2}$ max was 55 119 % of leg $\dot{V}_{O_2}$ max with an average of 85% $\dot{V}_{O_2}$ max during combined arm plus leg work was in average 6% higher than leg $\dot{V}_{O_2}$ max (range 99-117 %) It is demonstrated that there exists a rectilinear relationship between the arm $\dot{V}_{O_2}$ max and the combined V_{O_2} max when both are expressed relative to leg $\dot{V}_{O_2}$ max Work output during combined arm plus leg work was in all subjects higher than during leg work Aerobic and anaerobic metabolism contributed to this both being rectilinear related to the increase in work output, $\dot{V}_{O_2}$ max measured during leg and arm plus leg exercise was similar to that measured during bicycling and treadmill running respectively

Lammert, O (Inst for Physical Education Odense University Denmark THE EFFECT OF 3o MINUTES OF WORK WITH A CONSTANT HEART RATE (7o%) 0 1 2-3 4 5-6 or 7 TIMES A WEEK 99

The purpose of this investigation was to determine the relation between the effect of training and the training frequency when training intensity and duration were kept constant The training frequency was either 0 1-2 3 4-5 6 or 7 times per week for 4 weeks The training intensity was 7o% of the aerobic power and the training duration was 3o min After 4 weeks of training all subjects continued the training program 1 or 2 times per week for 8 weeks 7o% training intensity was defined as the workloads on a bicycle ergometer producing a constant HR = [7o%(max HR - rest HR)+rest HR] Before and at the end of 4-8 and 12 weeks training a double control was performed on each of the subjects The control consisted of 2 submaximal and 1 maximal workload on a bicycle ergometer For each workload, HR blood lactate concen tration and oxygen optake were measured

The effect of training determined as the increase in training workload in maximal oxygen optake in maximal ml O_2/kg/min and the decreased HR and blood lactate concentration at 900kpm/min was the same for a training frequency of 3 4 5 6 or 7 times per week A training frequency of 2 times per week had less training effect than 3 times per week The effect of 8 weeks of training at frequency of 1 or 2 times per week showed no systematic changes The conclusion is that a training frequency of 3 times per week has a maximal effect at the intensity of 7o%

trrame, S B and Aakvaag A (Laboratory of Physiology Norwegian College of Physical Education and Sport and Hormone nd Isotope Laboratory Aker Hospital Oslo Norway): EFFECTS OF ANDROGENIC ANABOLIC STEROIDS ON STRENGTH DEVELOPMENT AND PLASMA TESTOSTERONE LEVELS IN NORMAL MALES 100

Androgenic-anabolic steroids have been used xtensively by thletes to increase muscular strength and body weight However few controlled studies of the effect of such steroids on strength development have been done and the ignificance of the steroids in the training progress i still a controversial issue Furthermore no study has been reported regarding the consequenc of steroid treatment on pituitary function in normal males

To examine these effects physically homog neous group f 22 healthy males age 21 to 28 participated in a 8 week progressiv weight training program during which half of the group received the active drug on a double-blind basis (Mesterolone) i doses of 75 and 150 mg/day respecti ely for the first and last 4 week period All the subj cts were given ca 30 g supplemental prot in daily The maximal strength (isometric) of four muscle groups was tested

Although the steroid group showed gains over the controls in strength development the difference between the groups was not significant Highly significant suppression of plasma testosteron l els was observed in the steroid group the suppression being most pronoun ed during the high dos administration

If it is assumed that the plasma testosteron level reflect pituitary gonadotropin production the results suggest that thi androgenic anaboli steroid suppresses the pituitary

101 Holbech S & S H Johansen (Danish National Association for Infantile Paralysis and the Department of Anesthesia II Gentofte Hospital 2900 Hellerup Denmark): ENDURANCE TIME IN SLOW AND FAST CONTRACTING MUSCLE GROUPS

The endurance time (the maximum duration of a sustained isometric muscle contraction) of a slow and a fast contracting muscle group was investigated in 6 subjects The muscle groups examined were the plantar and the dorsal flexors of the ankle joint The measurements were made with a 90° flexion in the knee joint After determination of the maximum isometric muscle strength the endurance time was measured at 50 per cent of the maximum strength The plantar flexors showed a statistically significant longer endurance time than did the dorsal flexors This indicates that the ability of a muscle group to maintain a sustained isometric contraction depends on the relative proportion of slow and fast contracting muscle fibres

102 Karlsson, J. (Dept of Physiology GIH Stockholm, Sweden): ISOMETRIC LEG EXERCISE AND GLYCOGEN DEPLETION IN FAST TWITCH (FT) AND SLOW TWITCH (ST) FIBRES IN HUMAN SKELETAL MUSCLE

Isometric tension in skeletal muscle is proportional to the number of r units activated Activation of motor units with different contractile and metabolic characteristics might explain the differences in endurance time and metabolic response at different levels of isometric tension The present study was undertaken to determine the relationship between isometric tension and glycogen depletion patterns in human skeletal muscle fibres in an attempt to obtain some information about the type of fibres used at different tensions Biopsy samples were taken from the vastus lateralis muscle of subjects at rest during and after varying programs of isometric work Isometric tensions studied ranged from 10 to 40% of maximal voluntary contractile strength (MVC) of the knee extensors with the knee joint at 90° Muscle glycogen was determined chemically and estimated histochemically with the PAS technique At all tensions studied total glycogen declined but glycogen stores never were completely exhausted At tensions below 20% of MVC a preferential glycogen depletion occurred in the ST (high-oxidative) fibres When the tension exceeded 25% of MVC the FT (low-oxidative) fibres were the only fibres to become depleted of glycogen It was concluded that at low isometric tensions in skeletal muscle only ST fibres were recruited At higher tensions both fibre types were probably recruited with the FT fibres being the first to become depleted of their glycogen under these conditions

Piehl K P D Gollnick and B Saltin (Dept of Physiology GIH Stockholm, Sweden) SELECTIVE GLYCOGEN DEPLETION IN MUSCLE FIBRES DURING PROLONGED EXERCISE 103

A constant glycogen breakdown occurs in skeletal muscle during exercise Whether this occurs more rapidly in one or the other fibre type may depend upon the relative work load or on the speed for doing the work In the present study glycogen depletion patterns in human skeletal muscle fibres were followed during bicycle exercise with a pedal frequency of 60 rpm and loads requiring 30 60 or 90% of $\dot{V}_{O2}$ max Work was performed for 3 hr 2 hr or to exhaustion at 30 60 and 90% $\dot{V}_{O2}$ max, respectively Total glycogen was determined chemically and estimated histochemically in biopsy samples from the vastus lateralis muscle taken at rest during and after exercise Glycogen degradation was 2 7 and 7 4 times faster when working at 60 and 90% $\dot{V}_{O2}$ max, respectively than at 30% Slow twitch (high-oxidative) fibres were the first to lose glycogen (PAS stain) at all work intensities As work continued there was a progressive glycogen depletion in the fast twitch (low-oxidative) fibres Considerable quantities of glycogen remained in the muscle after 3 hr of work at 30% of $\dot{V}_{O2}$ max This was found almost exclusively in the fast twitch fibres Decreasing the pedal frequency to 30 rpm or increasing it to either 90 or 120 rpm while keeping total work output constant had little effect on the glycogen depletion pattern These data demonstrate that the pattern of glycogen depletion in human skeletal muscle fibres during bicycle exercise is more dependent upon the relative (% of $\dot{V}_{O2}$ max) than absolute work load or the pedal speed for doing a constant amount of work They also suggest a primary reliance upon slow twitch fibres during prolonged exercise

Saltin B, B Essén and B, Pernow (Dept of Physiology GIH Stockholm, Sweden and Dept of Clinical Physiology Huddinge Sjukhus Huddinge Sweden): LOCAL FACTORS ENHANCING MUSCLE GLUCOSE UPTAKE DURING EXERCISE 104

The extent that blood-borne or local factors influence glucose uptake during exercise is unclear This study was undertaken to analyze the effect of different muscle glycogen concentrations in the legs while work output and the supply of blood constituents were the same Six men performed 2-leg exercise at 2 work loads demanding 2 6 and 3 5 l/min (63 and 85% of $\dot{V}_{O2}$ max) for 20 min each or to exhaustion twice with 1 hr of rest between. Due to one-leg bicycle exercise the day before and consuming a fat and protein diet different amounts of glycogen were available in the thigh muscle of the 2 legs at the start of the experiment (65 (a) vs 35 (b) mmoles/kg) During 1 hr of rest between exercise sessions nicotinic acid was given to reduce the availability of FFA After the first work bouts muscle glycogen was reduced to 18 (a) and 7 (b) mmoles/kg At the end of the experiment only 3-5 mmoles/kg were left From the start of exercise there was a net uptake of glucose in both legs averaging 0 05 (a) and 0 09 (b) mmoles/l and during exercise a 8- and 5-fold increase was observed, respectively This occurred inspite of a reduction in arterial glucose to 2 1 mmoles/l Glucose uptake was inversely related to muscle glycogen content and the percentage of glycogen filled fibres Muscle glucose and G-6-P concentrations were lower in the leg with the lowest glycogen It is concluded that the most rapid glucose uptake during exercise occurs in the muscle (fibres) with a low glycogen content It is further suggested that this is brought about by a reduced local inhibition of hexokinase activity

105 Lindholm, A. (Dept of Physiology GIH and Dept of Clinical Biochemistry Royal Veterinary College Stockholm, Sweden) METABOLIC RESPONSE AND MUSCLE METABOLITES DURING DIFFERENT EXERCISE INTENSITIES IN TROTTING HORSES

Horse racing is an important industry and popular recreational activity The race horse is bred specifically for fast trotting and is a highly trained athlete At present very little information is available about the metabolic response of these animals to exercise of different intensities and durations The purpose of the present study was to describe changes in heart rate muscle temperature and muscle concentrations of glycogen, triglycerides lactate ATP and CP in the trained trotting horse after varying regimens of exercise Heart rate at rest was 36 beats x min^{-1} and it increased to 160 when the horse entered the track During maximal trotting it reached 230-240 beats x min^{-1} The muscle temperature at rest was 36 9 C which gradually increased to 41-42 C after several bursts of trotting Rectal temperature increased to as high as 40-41 C No marked change in muscle lactate occurred until trotting speed was within 10-15 seconds of maximal trotting speed (1 20 min x km^{-1}) Values as high as 25 mM x kg^{-1} wet muscle were recorded A marked muscle glycogen and triglyceride depletion was also observed However the energy stores are never completely depleted ATP and CP decreased in relation to the work load but the phosphagens never become depleted The horse was found to be a good species for metabolic studies during exercise and it seems highly possible that the local increase in muscle lactate and/or muscle temper ture limits the capacity for continuation of fast trotting

106 H P Hlastala B Wranne and C J Lenfant (Department of Medicine University of Washington Seattle Washington 98195 and Natl Heart and Lung Inst of Health, Bethesda Md 20014, U S A): PERIODIC HANGES IN FUNCTIONAL RESIDUAL CAPACITY AND OTHER RESPIRATORY VARI-ES IN RESTING MAN

Periodic changes in oxygen uptake measured at the mouth do not necessarily reflect changes in alveolar oxygen exchange They may also be due to changes in functional residual capacity (FRC), tidal volume (V_T) or respiratory period (T_R) This study was undertaken to deter mine the extent of such influences

Breath-to-breath changes in FRC VO_2 VCO_2 V_T T_R and alveolar gas tension were monitored in resting recumbent male subjects Changes in FRC were recorded using a body plethysmograph

FRC showed an oscillating pattern with from two to seven predominant frequencies The major oscillation had a period varying between 8 3 and 28 minutes from subject to subject with an amplitude varying from 42 to 176 ml Other major oscillations were present at higher frequencies with lower amplitudes Oscillations in $\dot{V}O_2$ $\dot{V}CO_2$ V_T T_R PAO_2 and $PACO_2$ were less marked and with less distinct frequencies $\dot{V}O_2$ and $\dot{V}CO_2$ were shown to be dependent on both FRC and V_T in terms of random changes but no strong relation was found in terms of periodic oscillations

We conclude that a true steady state of FRC does not exist This will influence breath-to-breath measurements of other respiratory variables such as $\dot{V}O_2$

Karvonen J (Department of Physiology University of Oulu Finland) WARM-UP IN LONG DISTANCE RUNNING AND SKIING 107

A warm-up program is performed by sportsmen empirically before a competition To elucidate the suitability of these procedures a study was made on 11 top-ranking runners and 14 mast r class skiers

A 10000 m race was run on two successive winter days with and without the warm-up period After the warm-up the mean result was better in seven cases (difference 18 to 160 sec) lower in one case and equal in three cases In 10 cases out of 11 the runners estimated that it was easier to run after a warm-up

Contradictory results were obtained in skiing experiments as a result of changing snow conditions In a 12 min skiing contest a better result was reached without a warm-up in 12 cases out of 14 The subjective estimation of the value of the warm-up was in agreement with the result only in four cases In a 15 km skiing contest no differences were observed when the time used for the first and second 5 km distance were compared as a function of the warm-up Subjective feelings were in favour of warming up

Warming up seems to include both a physical and a psychological component Too little is known as yet about its most effective methods and dosage

Gollnick, P, D, (Dept of Physiology GIH Stockholm, Sweden) 108
PHOSPHORYLASE. ROLE IN THE REGULATION OF GLYCOLYSIS DURING EXERCISE IN MAN

Peak rates of lactate formation in human skeletal muscle during intense dynamic or isometric exercise can be in the order of 0 4–0 5 x 10^{-4} moles x g^{-1} x min^{-1} The question has been raised as to how the glycolytic rate is regulated under these conditions The present study was undertaken to define the role for the conversion of phosphorylase (PLASE) b to PLASE a in human skeletal muscle during exercise Muscle biopsies from the vastus lateralis were taken at rest during and immedi tely after exercise and quickly frozen in liquid nitrogen PLASE was extracted from pulverized muscle samples in a glycerol- aqueous medium at temperatures below –25 C PLASE activity was analyzed fluorometrically from the production of G–1–P in the presence (PLASE a b) or absence (PLASE a) of 5–AMP (2 mM) Total PLASE (a b) activity ranged from 0 5–2 3 x 10^{-3} moles x g^{-1} x min^{-1} It was lowest in the muscles from endurance trained athletes and in muscles containing high concentrations of slow twitch fibres The PLASE activity was however at least 10–fold higher than peak rates of lactate formation At rest th ratio of PLASE a / (a b) was 17% Only small changes were observed following isometric or dynamic exercise In control experiments however electrical stimulation elevated th PLASE a activity to 25–30% in rat skeletal muscle It was concluded from these experiments that und r th conditions studied no major conversion of PLASE b to PLASE a took place These data suggest that the regulation of glycolysis in human skeletal muscle during exercise may be the result of some factor other than the conversion of PLASE b to PLASE a

109 Mäkäräinen M , Hirvonen L and Peltonen T (Department of Physiology University of Oulu, Finland) LEG AND ARM EXERCISE TESTS IN CHILDREN

To study the development of physical fitness in children separate leg and arm exercise tests using a Werding ergometer (Ergomat W J Werding Lausanne) were performed in sitting position on 30 boys and 16 girls from an elementary school's fifth and sixth grades (mean ages 11 ½ and 12 ½ years respectively) A conventional bicycle ergometer test served as a control In all tests the load was increased by 75 kpm/min at one minute intervals until exhaustion

The maximum heart rate in the bicycle ergometer test was the same for both sexes and grades (188 per min) In the leg test with the Ergomat exhaustion was reached in a shorter time and with a smaller load than in the bicycle test The maximum heart rate was about 25 beats per min lower in the Ergomat test The increase in the systolic arterial pressure was equal in both types of leg test In arm test the exhaustion stage was reached with a total work 10 to 15 per cent of that observed in the bicycle test The maximum heart rate was lower than in the leg test with the Ergomat

In the results for the fifth grade pupils no essential sex differences were observed The sixth grade girls reached the exhaustion stage with a lower load than the boys

110 Andén N E Magnusson I and Stock G (Department of Pharmacology University of Göteborg Sweden): BLOCKADE OF THE NERVE IMPULSES IN THE NIGRO-NEOSTRIATAL DOPAMINE NEURONS BY GAMMAHYDROXYBUTYRIC ACID AND GABA

The dopamine in the rat forebrain increased by about 70 per cent in 1 h both after axotomy and treatment with gammahydroxybutyric acid (sodium form 1 5 g/kg i p) Injections of 25% KCl but not 20% NaCl into the neostriatum prevented these increases in dopamine probably due to depolarization of the dopamine nerve terminals. Such a treatment with KCl also rapidly released the accumulated dopamine Intrastriatal injections of tetrodotoxin caused an increase in brain dopamine similar to that seen after axotomy

Injections of 25% KCl but not 20% NaCl into the substantia nigra induced an increase in brain dopamine to about the same degree as after axotomy This effect may be due to a persistent depolarization of the dopamine cell bodies and a consequent blockade of the nerve impulse flow in the nigro-neostriatal dopamine neurons Intranigral but not intrastriatal injections of GABA or gammahydroxybutyric acid also caused a marked increase in dopamine The analogue betahydroxybutyric acid was ineffecti e Gammahydroxybutyric acid may act by di ectly or indirectly mimicking an inhibitory GABA mechanism on the dopamine cell bodies i the substantia nigra.

Fuxe K Corrodi H Goldstein M Hökfelt T Lidbrink P and Unger- 111
stedt U (Department of Histology Karolinska Institutet 104 01 Stockholm
Sweden and Department of Psychiatry New York Medical Center New York
U S A): NEW TYPES OF DOPAMINE RECEPTOR STIMULATING AGENTS

With the help of biochemical histochemical and functional studies on the nigro-neostriatal dopamine (DA) pathway in rats it has been possible to discover that 1-(2 -pyrimidyl)-4-piperonylpiperazine (ET-495) and ergot alcal ids such as ergocornine and 2Br-α-ergocryptine (CB-154) ar DA rec[illegible] tor stimulating agents with a prolonged action and could be new tools in the treatment of Parkinson s disease These drugs induced a turning be[illegible] viour towards the intact side in rats with a unilateral 6-OH-DA induced de[illegible] gener tion of the nigro-neostriatal DA pathway The effects were bloc[illegible] DA receptor blocking agents and reduced by a tyrosine hydroxylase [illegible] suggesting some dependency of presynaptic DA stores Studies in r[illegible] with tremor following ventromedial tegmental lesions also show that [illegible] and CB-154 can cause reli f of tremor activity The studies on DA tur[illegible] suggested a dose-dependent decrease of turnover in the neostriatum [illegible] limbic forebrain following ET-495 ergocornine and CB-154 treatment [illegible] supports the view that they ar DA receptor stimulating agents

Recent exp riments with intraneostriatal injections of [illegible] nosine-3 5-cyclic phosphate demonstrate a turning of the rat towards [illegible] intact side an effect which was not blocked by DA receptor [illegible] These results support the idea that activation of the DA receptor [illegible] in the formation of cy lic AMP which causes an activity change in [illegible] effector cell

Avemo A Antelman S and Ung r tedt U (Dept of Histology [illegible] Institutet Stockholm Sweden): ROTATIONAL BEHAVIOR AFTER [illegible] CORTEX LESIONS IN THE RAT

Amphetamine (1-5 mg/kg) induces rot tional behavior [illegible] ed side in r t wher th nigro-striatal dopamine (DA) [illegible] rally lesioned with 6-OH-DA Apomorphine (0 25-2 mg/kg) [illegible] in the opposite dir ction Amph tamin probably releases [illegible] striatum whil apomorphine timul te supersensitive [illegible] nervated striatum (Ung rstedt 1971 Acta physiol scand [illegible] 93) In an attempt t study th functional interconnection [illegible] rats wer unilat rally lesioned in th frontal cortex [illegible] sions ar known to potentiat amphetamine excitation [illegible] exptl Therap 134 214) Unilater lly lesioned rats [illegible] inta t id aft r amphetamine (2 mg/kg) (releasing [illegible] sioned side aft apomorphine (2 mg/kg) (stimulating [illegible] results may be explained by an increased turnover of [illegible] frontal lesion which is further pot ntiated by [illegible] to d sensiti the DA receptors thus making [illegible] the oppo ite sid

113 Christensen A V (Department of Pharmacology and Toxicology H Lundbeck & Co A/S Copenhagen Denmark): ACUTE AND DELAYED EFFECTS OF A SINGLE DOSE OF A NEUROLEPTIC DRUG

In mice neuroleptics are known to antagonize methylphenidate-induced stereotypy (Pedersen V and A V Christensen Acta Pharmacol et Toxicol 1972 31 484) and thymoleptics have been shown to intensify apomorphine-induced compulsive gnawing (Pedersen V Br J Pharmac 1968 34 219P)

In the present study the effects of single doses of flupenthixol were followed over a period of 8 days Every day after flupenthixol treatment groups of mice were tested according to the above mentioned methods Complete or partial methylphenidate antagonism was recorded 1-2 days after flupenthixol administration While not inducing compulsive gnawing in normal mice apomorphine did so 2-5 days after flupenthixol

If methylphenidate antagonism is taken as an index of dopamine receptor blockade and the effect of apomorphine-induced behaviour as an index of receptor sensitization it may be concluded that a single dose of a neuroleptic drug causes a series of events in the neurone-receptor system initially characterized by receptor blockade and then followed by a receptor supersensitivity Thus the effect of a single neuroleptic dose appears to be more complex and longer lasting than usually thought

114 Kehr W Carlsson A and Lindqvist M. (Department of Pharmacology University of Göteborg Sweden): EFFECTS OF CEREBRAL HEMISECTION ON FOREBRAIN CATECHOLAMINE SYNTHESIS

As previously reported interruption of the nerve impulse flow of the dopaminergic l pathway results in a rapid and pronounced but short lasting increased rate of tyrosine hydroxylation followed by an increased accumulation of dopamine (Kehr W et al J Pharm Pharmacol 1972. 24 744) The effects of axotomy were further studied in different brai regions

Axotomy was performed by means of a complete transverse cerebral hemisection in rats at the level of the caudal hypothalamus. Noradrenaline dopamine tyrosine and dopa were measured after injection of NSD 1015 (3-hydroxybenzyl hydrazine HCl 100 mg/kg i p) an inhibitor of the aromatic amino acid decarboxylase When measured directly after hemisection the accumulation of dopa on the lesioned side was most pronounced in the striatum while the levels in the cortex and limbic system were only slightly elevated if compared to the intact side Two hours after hemisection the dopa levels on the lesioned side declined more rapidly in the limbic than in the striatal area however an increase was found in both regions on the i tact side After 1 3 days the tyrosine hydroxylation rate on the lesioned side declined even below control values accompanied by a drastic f ll i dopamine concentration

It is concluded that axotomy of dopaminergic neurones gives rise to an increase i tyrosine hydroxylation in the lesioned forebrain which may be due to a receptor-mediated feedback mechanism

Ahl nius,S Andén,N,-E, and Engel,J (Department of Pharmacology Univ rsity of Gothenburg Sweden) : RESTORATION OF LOCOMOTOR ACTIVITY IN MICE BY SMALL DOSES OF L-DOPA AFTER SUPPRESSION BY ALPHA-METHYLTYROSINE BUT NOT BY RESERPINE 115

The effects of low and high dose of L-DOPA on locomotor activity in mice were investigated after pretreatment with alpha-methyltyrosine or with reserpine After pretreatment with alpha-methyltyrosine the administration of low doses of L-DOPA (10 25 or 50 mg/kg i p , after inhibition of the peripheral L-DOPA decarboxyla e) restored the locomotor activity at 3 and 4 h concomitantly with a normalization of the brain catecholamine concentrations On the other hand the same treatments with L-DOPA did not cause any reversal of the reserpine-induced suppression of locomotor activity despit a marked increase in brain dopamine Both after pretreatment with alpha-methyltyrosine and with reserpine high doses of L-DOPA (?00 or 400 mg/kg i p) induced a marked increase in locomotor activity even in comparison to aline-treated controls and other signs of overstimulation The differen e between the fun tional effect of the low doses of L-DOPA after pretreatm nt with alpha methyltyro ine and with reserpine may be explained by a relea e of the newly form d catecholamine by nerve impuls s from the nerve terminals only in the former case

Aquilonius,S-M Eckernäs,S-Å. and Sundwall,A (Dept of Neurology and Dept f Pharmaceutical Pharmacol Univ of Uppsala Sweden):TOPICAL LOCALIZATION OF CHOLINE ACETYLTRANSFERASE(ChAc) IN HUMAN EXTRAPYRAMIDAL NUCLEI 116

Indirect evidence suggest that alt red central ch linergic function is involved in parkin onian symptoms and borea(Duvoisin R C Arch Neurol 1967 17 124;Aquilonius S-M &Sjöström R. Life Sci 1971 10 405) As the extent of cholinergic innervation f a brain region hould be r flected in its ti-vity of ChAc a d tailed knowledge f the ChAc distribution in human brain seemed of gre t importance This report concerns the topical localization of the enzyme in brains d rived 3-8h s post mortem from patients with no hi t ry of neurol gical disease

The caudate-thalamus-complex was di sected in six standardized frontal sections from the caudate head to nucleus(n)ruber and 3-5 mg specimens were punched out in about sixty loc lizations Enzyme activity (nmol s acetylcholine/g min) was det rmined by a modification f the radiochemical method described by Fonnum(Biochem J 1969 115 465)

The highest activitie we e found in putamen(186+8 7 n=28)and n caud tu (131+4 6 n=42) A significant difference was f und between lateral(17+2 0 n=9)and medi l(6+1 0 n=7) globus pallidus In the aff rementioned structure th ostro-caudal distribution of ChAc was rath even In the rost al s ction f the n caudatus howeve significantly lower activities war found than in the the l cali ations in this nu l ChAc activiti were slightly higher in th lamic nucl i(17_1 9 n=11) than in n ruber(10+0 6 n=3) substanti nigra(14_1 7 n=11)and n.hypothalamicus(13+1 3 n=2) Studi s are now extended to b ains l sioned by extrapyramidal diseases

117 Ljungberg T and Ungerstedt U (Department of Histology Karolinska Institutet Stockholm Sweden): 6-OH-DA INDUCED DA LESIONS: ADIPSIA APHAGIA SENSORY NEGLECT AND PHARMACOLOGICAL REVERSAL.

Bilateral lesions of the lateral hypothalamus induces a well-known syndrome of adipsia and aphagia (Anand and Brobeck 1951) Ungerstedt (1971) found the same syndrom after 6-hydroxydopamine induced lesions of the nigro-striatal dopamine (DA) system and postulated that the lateral hypothalamic syndrome was due to lesions of the DA fibres to the striatum and not to lesions of an "eating-centre in the lateral hypothalamus We have now tried to substitute the lost DA by giving 6-OH DA denervated animals the DA receptor stimulating drug apomorphine Bilateral DA lesions abolishes previously learned maze running for water reward After a low dose of apomorphine the animal runs the maze and drinks the water Furthermore a lesion of the DA pathway gives a sensory neglect to tactile olfactory visual and auditory stimuli on the side of the body contralateral to the lesion This disability to orient towards sensory stimuli probably explains the adipsic and aphagic syndrome The same sensory neglect has been found after lateral hypothalamic lesions (Marshall et al 1972) The reversal of adipsia and aphagia after apomorphine further supports the DA hypothesis

118 Lorens, S A and Yale, H. (Institutes of Psychology and Physiology, University of Bergen, Norway): DISSOCIATION OF THE ANALGESIC AND REWARDING EFFECTS OF INTRACRANIAL STIMULATION

Bipolar electrodes were implanted in the lateral hypothalamus (n=10) or medial frontal cortex (n=10) of rats The animals were trained to press a lever to deliver intracranially sec trains of square waves Self-stimulation current thresholds were obtained, and the animals tested at this current value 10 min /day for 10 consecutive days The effect of stimulation on pain reactivity then was determined by measuring paw lick or jump latency following placement on a hot plate (55 C) The absence of either behavioral response for 45 sec indicated an analgesic effect of the stimulation

Stimulation of the lateral hypothalamic area produced both rewarding and analgesic effects The current intensity necessary to produce analgesia, however, was significantly greater than that required to maintain self-stimulation behavior Stimulation of the medial frontal cortex also yielded a positively reinforcing effect, but failed to induce analgesia regardless of the intensity or rate of stimulation administered Stimulation of a brain site which yields a positively reinforcing effect thus will not necessarily induce analgesia This suggests that different anatomical systems are involved in mediating the rewarding and analgesic effects of intracranial stimulation

Knapp, T H Lubar J F , (Dept of Psychology Univ of Houston Houston Tx 77004; Dept Psych ,Univ of Tenn Knoxville Tn p t Institute of Psychology Bergen): OPERANT CONDITIONING OF 40 HZ ACTIVITY IN THE AMYGDALOID NUCLEI OF THE CAT 119

High frequency activity such as 40 Hz in the amygdala has been associated with olfactory functions (Gault and Leaton Electroenceph clin Neurophysiol 15 1963),emotional states (Leese Psychiat Res Repts 12,1960),and arousal (Delgado et al ,Brain Res 22 1970) Using cats with chronically implanted bipolar electrodes located in the basolateral amygdala and medial forebrain bundle (MFB) each animal was reinforced for the occurrence of 40 Hz amygdaloid activity Reinforcement consisted of MFB stimulation (60 Hz, 0 5 sec 0 2-0 8 ma) beginning with a CRF schedule and progressing to a FR 12 schedule After 56 days of training 30 min per day the cats showed a sixfold increase over initial 40 Hz levels These animals were also capable of discriminating the differential contingencies under which they were trained Following completion of this task the cats were extinguished until their preconditioning baselines were recovered Correlates of increased 40 Hz amygdala activity included aggressiveness as manifested in difficulty in handling the animal by the experimenter altered emotionality and EEG time-period changes

Supported by USPH-NIMH Training Grant ##MH 10513-07 to J F Lubar

Livesey P.J & Meyer P   (Psychology Department University of Western Australia): LEARNING A BRIGHTNESS DISCRIMINATION TASK UNDER CONDITIONS OF ELECTRICAL STIMULATION OF THE DORSAL HIPPOCAMPUS IN THE RAT 120

Rats with electrodes in the CAI area of the dorsal hippocampus given 0 5 sec train of electrical stimulation every 3 0 sec during training on a brightness discrimination test failed to learn   Following learning without stimulation such stimulation led to a significant decline in correct responses   With 2 0 sec of stimulation 5 0 sec after each response learning was gain disrupted but the stimulation did not affect performance after learning was achieved   This sugge ted interference with both consolidation and retrieval processes (LIVESEY P & WEARNE G   Neuropsychologia 1973 11 75)

T further explore this possibility 3 groups each of 9 rats were trained on the task   One group was stimulated only during the period of exposur to the stimuli the second group was given timulation 10 sec after response and the third group a control group with electrodes in the cortex directly over the CAI area was given 0 5 sec of stimulation every 3 0 sec during training   Group 1 animals showed disruption of learning and Group 2 animals showed some retardation in the rate of a quisition   All animals were brought to criterion and then were tested under 3 different stimulation conditions i e "continuous stimulation   timulation during stimulus presentation and stimulation 5 sec after response   Disruptive effects were observed in some but not all animals under conditions 1 and 2 The nature of thes effects appeared to be dependent on the location of the electrodes in relation to the CAI area

121 Lidbrink, P (Dept of Histology Karolinska Institutet Stockholm Sweden): ON THE ROLE OF CENTRAL NA NEURONS FOR REGULATION OF SLEEP AND WAKING

The sleep-waking cycle can be altered by limited lesions to either noradrenaline (NA) or 5-hydroxytryptamine (5-HT) neurons (Jouvet M Science 1969 163 32) Rec nt pharmacological studies indicate that the NA neurons are of importance for wakefulness (Jones B Brain Res 1972 39 121) To test the relationship between wakefulness and different NA neuron systems specific lesions of the NA pathways continuous recording of the EEG and subsequent evaluation of changes in NA levels have been performed

Lesions of ascending NA axons from cell bodies in the brain stem were made by intracerebral injections of 6-hydroxydopamine The EEG and neck EMG was continouously recorded for 4-8 days after the l sion The recordings were scored for wakefulness and the different states of sleep The effect of the lesion on the NA neurons was evaluated with flu rescence histochemistry and biochemical d terminations of NA

Lesions which caused disappearance of the NA nerve terminals in the cerebral cortex produced a d crease of wakefulness by about 25% This time was replaced by a pattern of c rtical deactivation with slow waves The effect was more pronounc d when the l sion also included th NA axons projecting to subcortical areas in the forebrain Activation of the cortex during paradoxical sl ep was not ffected The animals gradually recovered from the loss of wakefulness d e to the lesion and after one month they showed a normal sleep-waking cycle

The r sults sugg st that NA neurons in the low r brain stem can directly influence the forebrain and are of importance for maintaining the activation f cortical ctivity d ring wakefulness

122 Ågren G and Meyerson, B J (Dept of Med Pharmacology University of Uppsala Sweden): PREFERENCE FOR PAIR PARTNER IN THE MONGOLIAN GERBIL

Male nd female gerbils were kept in pairs from about 90 days age and permitted at least one litter before the male was vasectomized The preference of the female for her male pair-partner versus a strange male was tested The pparatus onsisted of three goal-cages each connected t a entrally placed cage by means of registrating units which showed the locati n of th t st-animal One goal-cag held the pair-partner another one strange male and the third goal cage was empty The contact with the males was restricted by a wire-mesh The time spent in the different goal-cages was recorded during a t st-session of 3 hours The tested subject was isol ted for 3 hour before each test-session One test-s ssion was performed per day/ nimal for 5 consecutiv days

A preference f 72% was seen f r the pair-partner Castration f the p ir-p tne reduced the prefe enc whereas astration of the strange male did not significantly influenc the preference When the female was in di strus th preferenc was higher than in strus (cornified vaginal sme rs)

The pref rence of the male for his pair-partner was tested analogously nd wa related to the stage f estrous-cycle of both the pair and the str ge femal

Ahlenius S and Engel, J (Department of Pharma ... Gothenburg Sweden): A ROLE OF CENTRAL NORADRE... AVOIDANCE BEHAVIOUR

The administration of a dopamine beta-hydroxylase i... (bis (4-methyl-1 homopiperazinylthiocarbonyl)di... induced a partial disruption of a conditioned av... mice The CAR was suppr ssed to 75 per cent o ... r 24 h after the FLA-63 tr atment The same d... noradrenaline (NA) t about 35 per cent of contr... str tion and to about 30 per cent of contr l ... was a slight increase in brain dopamin (DA) ... administration

The administration f DL-threo-3 4-dihydroxy... i p antagonized the FLA-63-induced reductio... time (Zh) partly counteracted the decreas in ... caused a decrease in brain DA

From the pr sent results it is uggested th... the mediation of a CAR in mice

Buus Lassen, J (Department of Pharma... 2860 Soeborg Denmark): EFFECT OF P-CHLOR... MOTILITY IN RATS AFTER INHIBITION OF ... STORAGE UPTAKE AND RECEPTORINTERACTI...

PCA has been shown to deplete brain ... Tricyclic thymoleptics and chlorpheni... tagonise this 5HT-depletion (Squires ... toxicol. 1972 31 suppl 1 55) In the ... the eff ct of PCA on motility of rats ... studied using Animex motimeter ... hyperactivity consisting of abnormal ... head movements from side to side ... were also present

The ffect of PCA 5 mg/kg was invest... tion of substances interfering with ... The 5HT-synthesis inhibitor H 69/17 ... hydroxylase inhibitor H 44/68 250 mg/kg ... 7 5 mg/kg imipramine 3 1 mg/kg chlor... desipramine 26 mg/kg amitriptyline ... 1 7 mg/kg haloperidol 0 01 mg/kg and ... antagonized PCA-hypermotility H ... dopamine (DA) β-hydroxylase inhibitor ... influence the effect of PCA

These results indi ate that 5HT ... hyperactivity and that 5HT-membrane ... of PCA into brain 5HT neurons

125 Brown, R Engel J Carlsson A (Department of Pharmacology University of Göteborg Sweden): EVIDENCE FOR CATECHOLAMINE INVOLVEMENT IN THE DISRUPTION OF BEHAVIOR DURING HYPOXIA

Hypoxia results in a decreased hydroxylation rate of tyrosine and tryptophan (Davis and Carlsson J Neurochem 20:913 1973) Two experiments were performed to determine if the disruption of catecholamine (CA) metabolism was of behavioral importance In mice the depression of locomotor activity under 12% oxygen was reversed by administration of apomorphine plus clonidine These agents directly stimulate CA receptors The locomotor activity under hypoxia was not in reased by amphetamine a drug which is bel ived to act by releasing newly synthesized CAs However if a low dose of L-DOPA plus a peripheral decarboxylase inhibitor was administered prior to amphetamine the locomotor activity was stimulated To examine the effect f hypoxia on a more specific behavior rats were trained to av id grid shock in a shuttle box Avoidance responding was impaired by 8 65 % oxygen b t the blockade was reversed by administration of L-DOPA plus a peripheral decarboxylase inhibitor The data suggest that central CAs are involved in the behavioral disruption under hypoxia and that when transmitter precursor is supplied so that synthesis by-passes the oxygen-requiring rate-limiting step central dopaminergic and possibly noradrenergic neurons are able to maintain behavior

Persson, S-A and Wahlström, G (Department of Pharmacology University of Umeå Sweden): TUBE RUNNING ACTIVITY IN MICE TO EVALUATE THE EFFECTS OF DRUGS

The 5-Hydroxytryptophane (5-HTP) syndrome in mice includes hyperextended abducted hindlegs The movements of the hindlegs are ineffective on a flat surface This part of the syndrome has been used in a simple quantitative test

One hour prior to the test male mice (25-33 g) were put in a 120 cm plexi-glass tube with an inside diameter of 3 2 cm At the time of testing a gentle air stream was started blowing in the direction of the run The mice were placed in the air inlet end of the tube and the time it took for the mice to run 100 cm was measured

In preliminary experiments mice pretreated with Nialamide (50 mg/kg i p) 2 h prior to 5-HTP (50 mg/kg i p) were tested every 15 minutes for 3 h after the last injection They consistently passed the tube faster than Nialamide-saline treated controls (for instance 9 00±1 51 (SE) s N = 6 versus 129 5± 49 6 s N = 6 t = 2 43 P < 0 05 two hours after the last injection) Pretreatment with lithium chloride (5 meq/kg i p 20 min prior to the 5-HTP) decreased the effect of 5-HTP (64 7±18 4 s N = 6 t = 3 02 P 0 02)

In mice the 5-HTP syndrome thus seems to facilitate the ability to pass through a tube

Ranje Ch and Ungerstedt U (Dept of Psychology University of Stockholm Sweden and Dept of Histology Karolinska Institutet Stockholm Sweden): EFFECTS OF CHRONIC AMPHETAMINE AND APOMORPHINE UNDER CONDITIONS OF HIGH MOTIVATION 127

Ten rats were fully trained to escape an underwater Y-maze by choosing the illuminated instead of the dark arm Illumination was randomly changed between arms The effect of chronic administration of d-amphetamine (6mg/kg 40 min before swimming) or apomorphine (5mg/kg 5 min before swimming) was investigated by testing for swimming time and errors after these drugs every second day and without drugs every other second day Amphetamine treated animals fell into three groups on the basis of swimming time: animals unaffected by the drug animals using 6 times longer and animals using 17 times longer swimming time than normals There was an insignificant increase in number of errors The apomorphine treated animals responded as a homogenous group using 9 times longer swimming time than normals They showed a significant increase of errors The different effects of amphetamine and apomorphine may be due to their pharmacological effects: amphetamine releasing dopamin and noradrenalin apomorphine stimulating dopamin receptors The individual differences among the amphetamine animals are interesting in terms of the addictive properties of the drug

Carrer H and Meyerson B J (Dept of Med Pharmacology Univer ity of Uppsala Sweden): INFLUENCE OF LSD APOMORPHINE AND MONOAMINE SYNTHESIS INHIBITORS ON THE URGE TO OBTAIN WATER AND SEEK HETEROSEXUAL CONTACT IN THE FEMALE RAT 128

To tudy the influenc of varying monoaminergic activity levels on motiva tion a serotonin agoni t (lysergic acid diethylamid LSD) a dopamine agoni t (apomorphine APO) and monoamine synthesis inhibit r (p-chl ophenylalanine PCPA and α-methyl-p-tyrosine MT) wer used The effect of these agents on the urge to drink wate in water deprived animals or to seek heterosexual contact in castrated estrogen treated female rats was determined Motivation was measured using the increasing barrier t chnique[1] i e th willingnes to cross an electrical grid to reach water or a exually tiv mal In parall l th influence on spontaneous motor tivity (SMA) f the different tr atments was r corded

LSD 0 05 mg/kg but not 0 01 mg/kg ignificantly d cr as d the number of crossing when the goal cag held a male The higher dose cle rly d pre d SMA Apomorphine (0 05 or 0 02 mg/kg) d creased the number of crossings f water Identical dos s f APO significantly depressed SMA

αMT 100 mg/kg decreased ros ings t reach male but slightly increased crossings for water PCPA was given f four cons cutive days (100+50x3 mg/kg) PCPA treatment incre sed grid crossings by females reaching fo the male; this effect was ob rved only in oestradiol benzoate tr ted subjects The ame tr tment decreased cros ings in water deprived animal seeking water

[1]Meyerson B J and Lindström L Acta Phy iol suppl 389 1973

129 Eliasson, M and Meyerson B J (Dept of Medical Pharmacology University of Uppsala Sweden): INFLUENCE OF LSD AND APOMORPHINE ON HORMONE-ACTIVATED COPULATORY BEHAVIOR IN THE FEMALE RAT

Increased monoaminergic activity decreases the estrogen+progesterone activated copulatory response (lordosis reflex) in female ovariectomized rats An antagonistic action of serotonin has been proposed based upon effects of neuropharmacological agents with different actions on central nervous monoaminergic mechanisms [1]

The present investigation is concerned with the effects on the female copulatory response of LSD and apomorphine Accumulating evidence suggests agonistic effects of LSD on central serotonin receptors and of apomorphine on dopamine receptors

LSD 0 1 mg/kg significantly inhibited the lordosis reflex 10 min after injection Higher doses had a more prolonged effect with complete recovery at 90 min The effect of LSD was reduced by α-m-Tyrosine 200 mg/kg 5 hrs before LSD and prolonged by p-chloro-phenylalanine 350 mg/kg 5 hrs before LSD Chlorpromazine 0 5 mg/kg 1 hr before did not influence the LSD effect but the dopamine antagonist pimozid 0 5 mg/kg 1 hr before reduced the inhibitory effect of LSD Apomorphine 0 20 mg/kg significantly inhibited the lordosis reflex 10 min later with complete recovery at 60 min Effect of a higher dose was not recovered until 120 min This effect was prevented by treatment with pimozid 3 hrs before but was not influenced by α-m-Tyrosine pretreatment The results will be discussed with regard to the implication of serotoninergic and dopaminergic mechanisms in the inhibition of hormone activated copulatory responding in the female rat

[1] Meyerson et al In: Neuropharmacol gy: Proc VIII C N I P Congr Copenhagen 1972 in press

130 Otto, U (ACO Läkemedel AB Box 3026 S-171 03 Solna 3 Sweden): THE ANALGESIC ACTIVITY AND ACUTE TOXICITY OF SODIUM SALICYLATE AND CODEINE PHOSPHATE COMBINATIONS IN RAT

The experimental animals were rats of Sprague-Dawley strain Sodium [illegible]cylate and codeine phosphate and combinations of these drugs were [illegible] subcutaneously

The analgesic activity was determined by means of a technique described by Paalzow (Acta Pharm Suecica 6 193 1969 & Ibid 6 207 1969) and later modified by Otto et al (To be published) In this method the tail of the rat is stimulated for 80 ms using a frequency of the electrical square waves of 125 Hz and a pulse-width of 1 6 ms A nociceptive response is assumed to be produced when the animal squeaks The pain threshold voltage for each animal is determined before administration of the drug After injection of the drug the threshold is registrated at 10 min intervals and the graded response is expressed as a percentage of the pre-treatment threshold voltage

The LD_{50} determinations were calculated at minimum three dose levels with at least three groups of rats The mortality ratio was recorded 24 h after administration

Results were obtained indicating that the analgesic effect of the combinations of sodium salicylate and codeine phosphate seemed to be additive The toxicity of codeine phosphate however was found to be potentiated of sodium salicylate while the acute toxicity of sodium salicylate was not influenced by an addition of codeine phosphate

Larsen J -J and Hermansen, K (Research Division of Phar- macia AS Hillerød Denmark): NEUROLEPTANALGESIA PRODUCED BY A COMBINATION OF ETORPHINE AND ACETYLPROMAZINE AND ITS REVERSAL BY AN ANTAGONIST DIPRENORPHINE IN THE DOG 131

The pharmacological actions of etorphine have been described previously by Blane G F et al (Br J Pharmacol 1967 30 11-22) Administration of etorphine and acetylpromazine in combination (Immobilon[R]) produces a clinical useful neuroleptanalgesia in the dog which is readily antagonized by diprenorphine (Hansen N H and Szabo J Nord Vet -Med 1973 25 38-45)

The analgetic effect of Immobilon (etorphine 125 µg/ml and a etylpromazine 500 µg/ml) has been studied and compared with that of its two components and morphine The following values were found (ED 50 µg/kg s c ; acetic acid test mice): Immobilon (etorphine 0 3 + acetylpromazine 1 2); etorphine 1 2; acetylpromazine 40 and morphine 350

The neuroleptanalgesia (0 05 ml/kg Immobilon i v) was evaluated by checking different reflexes in seven dogs At the ame time the cardiova cular action of Immobilon and its antagonist diprenorphine (Revivon[R]) was followed by recording the systolic/diastolic blood pressure the heart rate and the ECG It is concluded that the intravenou administration of th combination of etorphine and acetylpromazine results in a clinical useful neuroleptanalgesia without side effects from th cardiovas ular system prohibitive for its clinical use

Wahlström, G (Department of Pharmacology University of Umeå Sweden): THE RELATION BETWEEN SEIZURE AND TOLERANCE IN THE ABSTINENCE SYNDROM AFTER CHRONIC BARBITAL TREATMENT IN THE RAT 132

Seizures as a symptom of physical dependence could be one way for the organism to decrease excitability in the CNS; normali ze the functional state of the CNS Such changes in excitability may be revealed by changes in tolerance

Male rats were treated with barbital in the drinking water (consumption: 190-260 mg/kg/day) for 4 5 month The threshold dose of hexobarbital needed to obtain an EEG-criterion (Wahlström G Acta pharmacol toxicol 1966 24 404) was used as a measure of tolerance 3 days after the end of the barbital treatment Seizures were induced by electrical stimulation 1 h prior to the threshold determination The results (controls inside brackets) are given in per cent of a pre-experimental average

Experimental treatment prior to test	Number of thresholds below 110 %	above 110 %	Mean threshold per cent
Seizure	8[x] (18)	10 (0)	119 (90)
No seizure	0 (17)	13 (1)	141 (89)

x) One rat had a spontaneous seizure 0 25 h prior to test

A seizure could thus abolish tolerance in approximately 50 % of the barbital treated animals The hypothesis stated above has not been refuted by the present experiments

133 Malmnäs C O and Meyerson B J (Department of Medical Pharmacology Univ of Uppsala Sweden): EFFECTS OF AN ANTIESTROGEN MER-25 ON TESTOSTERONE-ACTIVATED COPULATORY BEHAVIOR IN THE CASTRATED MALE RAT

Male Wistar rats (purchased as Specific Pathogen Free) castrated as adults were maintained on a submaximal response level with respect to the percentage of subjects which displayed copulatory behavior with a receptive female (mount%) by means of testosterone propionate (TP 0 20 mg/kg/week s c) Copulatory tests were performed once weekly 2 days after the TP injection The results obtained after an experimental treatment were compared with the pre-experimental response level

An antiestrogen ethamoxy-triphetol (MER-25) 150 mg/kg given 2 hrs before the TP injection decreased mount% from 73% to 41% (p<0 001 N 37) discontinued TP treatment decreased mount% from 78% to 49% (p<0 001 N=37) while oil vehicle given 2 hrs before the TP injection did not affect the response Furthermore it was found that estradiol benzoate 50 µg/kg could activate copulatory behavior in never-TP-treated nonmounting subjects

The data are consistent with the hypothesis that testosterone is first converted to estrogen before copulatory activation is brought about

134 Buus Lassen J (Department of Pharmacology A/S Ferrosan 2860 Soeborg Denmark) EFFECT OF 4 α-DIMETHYL-M-TYRAMIN (H 77/77) ON MOTILITY IN RATS AFTER INHIBITION OF MONOAMINE [illegible]SIS UPTAKE AND RECEPTORINTERACTION

H 77/77 has been shown to deplete brain noradrenaline (NA) of rats and mice Tricyclic thymoleptics were found to antagonize this NA-depletion (Carlsson et al Europ J Pharmacol 1969 9 367-373) In the present investigation the effect of H 77/77 on motility of rats in a familiar cage was studied using Animex motimeter, 2 5 and 5 mg/kg s c induced hyperactivity consisting of locomotion sniffing rearing different grooming movements and head twitch Those behavioral items are almost identical to those observed in untreated rats during exploratory activity in an unfamiliar cage

The effect of H 77/77 5 mg/kg was investigated after s c treatment with substances interfering with central monoamine function The tyrosine hydroxylase inhibitor H 44/68 (250 mg/kg), the dopamine-β-hydroxylase inhibitor FLA 63 (40) imipramine (0 7) desipramine (6 3) amitriptyline (0 1), chlorimipramine (1 5) protriptyline (0 7) and doxepin (1 1) antagonized H 77/77-hypermotility The serotonin synthesis inhibitor H 69/17 (200) did not influence the effect of H 77/77

These results indicate that release of brain NA is involved in the H 77/77 induced hyperactivity and that NA-membrane blocking thymoleptics inhibit the uptake of H 77/77 into brain NA neurons

Persson E B Agerup J Schnermann (Institute of Physiology University of Uppsala Sweden and Institute of Physiology Munich GFR) 135
GLOMERULAR HYDROSTATIC PRESSURE AND LOOP PERFUSION RATE

To investigate the effect of changes in distal tubular sodium delivery on glomerular filtration the following experiments were performed on rats Glomerular capillary pressure was estimated from proximal tubular stop flow pressure (SFP) measured with a servo null recording system proximal to an injected highly viscous silicone oil block Distal to the oil block the late proximal segment was perfused either with Ringer or 300 mM mannitol solution at flow rates above and below the normal of about 13 nl/min When the distal nephron was perfused with Ringer zero flow SFP was 53 3 cm H_2O +5 2 (SD) and the decrease in SFP at 13 27 and 43 nl/min were 0 1 +0 5 7 0 +4 4 (p< 0 001) 10 5 +4 0 cm H_2O respectively When mannitol was the perfusing fluid there were no significant differences in SFP at different flow rates It is suggested that increase in distal sodium delivery gives rise to an increase in afferent arteriolar resistance whereupon the glomerular filtration rate decreases At reduced distal delivery the intrarenal regulation is either abolished or may involve changes in the resistance of both afferent and efferent arterioles

Agerup B (Institute of Physiology and Medical Biophysics University of Uppsala Sweden) THE EFFECT OF CHANGES IN THE PERITUBULAR PHYSICAL FORCES ON NET FLUID TRANSFER IN THE RATS KIDNEY PROXIMAL TUBULE 136

Changes in transtubular net fluid transport have previously been induced by altering the intratubular hydrostatic and oncotic forces These experiments seem to indicate a significant role for the existing physical forces on normal tubular reabsorption (A E G Persson et al Kidney Int, 1972, 2, 203) To test whether these findings were valid when alterations were made at the peritubular side of the nefron the following set of experiments were undertaken

Reabsorptive rate was measured from sequences photographs of a filtrate droplet enclosed between two oilcolumns in a straight surface proximal tubule The tubular surrounding was perfused in two different ways A Perfusion of the capillaries in the star vessel at 250 ml/min with ultrafiltrate plus 10 g% albumin B Perfusion of the subcapsular interstitium ultrafiltrate plus 10 g% albumin

The difference in transtubular fluid flow between control and perfusion expressed as nl/mm^2 sec was:

A : + 0 069 ± 0 065 p < 0 001 control: 0 405 ± 0 090 n = 16
B : + 0 012 ± 0 101 p > 0 5 control: 0 456 ± 0 096 n = 20

These experiments showed that an increased capillary oncotic pressure induced a significantly increased tubular reabsorption However an increased subcapsular interstitial oncotic pressure did not effect tubular reabsorption A direct tubulo-capillary transport route is consequently suggested

137 Røder M, Ouvik P, Jr, and Kiil, F, (Institute for Experimental Medical Research Ullevål Hospital Oslo Norway) MECHANISM OF MAINTAINED GLOMERULAR FILTRATION RATE AT HIGH URETERAL PRESSURE.

Glomerular filtration rate (GFR) is maintained when ureteral pressure is raised on account of afferent arteriolar dilatation On the other hand, GFR remains constant during a rise in renal perfusion pressure (RPP) on account of afferent arteriolar constriction To examine if the relationship between GFR and ureteral pressure is influenced by RPP the effect of ureteral obstruction on GFR was studied at two RPP in 6 anesthetized dogs RPP was elevated by carotid constriction GFR was estimated as 51-Cr-EDTA extraction

At control RPP averaging 127 ± 5 mm Hg GFR exceeded 91 ± 8% of control up to ureteral pressure of 45 ± 1 mm Hg At high RPP averaging 183 ± 4 mm Hg GFR exceeded 96 ± 2% up to ureteral pressure of 80 ± 2 mm Hg At ureteral pressures exceeding 50 mm Hg GFR was significantly lower ($p < 0\ 05$) at control RPP than at high RPP

Thus the vasoconstrictive effect on afferent arterioles induced by raising RPP can be partly compensated by raising ureteral pressure suggesting autoregulation of the transmural pressure of the afferent arterioles

138 Eide I Løyning E and Kiil F (Institute for Experimental Medical Research University of Oslo Ullevål Hospital Norway: THE EFFECT OF ATHETIC β-RECEPTOR STIMULATION ON RENIN RELEASE DURING REDUCED RENAL FUSION PRESSURE.

The mechanical stimulus for renin release during constriction of the renal artery appears to be autoregulated dilatation of afferent arterioles (Eide I Løyning E. and Kiil F Circul t Res 1973 32 237) Renin release may also be raised by stimulation of β-receptors but the interrelationship between the two types of stimuli remains unclear

In ten dogs intrarenal infusion of isoproterenol at 4.0 µg per min increased the secretion of renin from 1 6 ± 1 5 (mean ± SD) to 8 9 ± 5 3 µg per min ($p < 0\ 001$) Similarly, during standardized reductions of perfusion pressure below the range of autoregul tion of renal blood flow isoproterenol increased renin release by 70% from 31 9 ± 10 2 to 54 3 ± 15 7 µg per min ($p < 0\ 001$) This increase is larger than at control perfusion pressure ($p < 0\ 01$) Conversely β-receptor blockade with propranolol in five dogs reduced renin release by 38% from 47 6 ± 28 0 (range 25 1 – 93 7) to 29 5 ± 10 5 µg per min (reduced in four and unaltered in one dog) more

Thus stimulation of β receptors increases renin release/at low than at control perfusion pressure β-receptor activity became therefore an effective regul tor of renin release at reduced renal perfusion pressure

Lindbom, L-O, and H.R. Ulfendahl (Institute of Physiology and Medical Biophysics University of Uppsala Sweden) INFLUENCE OF BRADYKININ ON PROXIMAL TUBULAR REABSORPTION 139

The regulation of proximal tubular reabsorption of sodium is essential in maintaining water and electrolyte balance in the body Several attempts have been made to find substances that decrease sodium reabsorption in the proximal tubules Among the substances tested is bradykinin which has been shown to increase the urinary output of sodium This study was undertaken to find out whether the increased output is due to an effect on the proximal tubular reabsorption of bradykinin

On Inactin[R]-anaesthetized rats the left kidney was prepared for micropuncture A split-oil droplet technique was used to determine tubular reabsorption by time-sequenced microphotography From the photographs the t1/2 for the droplets was determined and used as a measure of tubular reabsorption Thereafter a cannula was inserted into an adjacent star vessel and a perfusion of the peritubular capillary network was started During this perfusion another determination of the tubular reabsorption was done In one series the perfusate was a modified Ringers solution in another series 0 9 micrograms per ml of bradykinin was added to the perfusate The perfusion rate varied between 400 and 900 nl/min Results *first series* control t1/2 18 3 sec perf t1/2 18 6 sec Second series control 18 6 sec perf 26 9 sec This increase of about 50% in t1/2 during bradykinin perfusion is interpreted as a direct influence of bradykinin on the proximal tubular reabsorption as compared to Ringer perfusions where no change was seen

Jorgensen, K E, J V Møller and M I Sheikh (Institute of Medical Biochemistry, University of Aarhus Denmark) 140

THE USE OF ^{14}C- AND ^{3}H-LABELLED DERIVATIVES OF INULIN AS TRACERS OF INULIN IN VIVO

Radioactive derivatives of inulin ($^{14}CH_2OH$- $^{14}COOH$- or C^3H_3O-inulin) were infused together with native inulin and creatinine into anaesthetized rabbits It was found that the renal clearance values of the labelled derivatives did not differ from those of unlabelled inulin and creatinine Furthermore the specific activity of plasma and urine was the same as that of the infusion fluid The plasma decay curves of radioactive and native inulin after withdrawal of the substances from the infusion fluid coincided and similar values were calculated for the distribution volumes of these substances (appr 26 %) By contrast the decline of plasma creatinine was slower than that of inulin and the distribution volume was found to be 45 % Gel filtration analysis indicated the presence of varying degrees of low molecular weight contaminants in the labelled derivatives of inulin It is concluded that in spite of the presence of impurities radioactive derivatives may be used as a substitute of native inulin for determination of glomerular filtration rate and extracellular space in the rabbit

141 Ulfendahl H R Pinter G G Atkins, J L Wolgast M and Agerup B (Inst of Physiol and Med Biophys Univ of Uppsala and Dept of Physiol Univ of Maryland Baltimore Md USA):
TOTAL LYMPH FLOW OF RAT KIDNEY

To determine whether the flow of lymph plays a significant role in the fluid balance of the kidney and to estimate the rate of lymphatic drainage of extravascular plasma proteins from renal tissue we have attempted to measure total renal lymph flow (TRLF) in rats We observed that a partial occlusion of the left renal vein (PORV) brought about an immediate and large increase in the flow of a main abdominal lymph duct (ALD) cannulated next to the aortic hiatus of the diaphragm Moreover we found that occasionally in Sprague Dawley rats small lymphatics of the renal hilum appeared to join together into a single major renal lymphatic vessel When lymph flow from such a vessel was diverted into a separate collecting cannula flow from ALD decreased and failed to respond to PORV Based on these observations in preliminary experiments we estimated TRLF either as the difference in ALD flow before and after complete occlusion of the renal hilum (O Morchoe and O Morchoe J. Physiol. /London/ 1968 194 305) or calculated it by using the responses to PORV that occurred simultaneously in flows of a cannulated renal hilar lymph vessel and ALD In rats weighing 200-350 gms that had received no fluid load we obtained a value of 2 to 3 µl/min/gm of kidney which we consider to be an approximate estimate rather than an accurate measure of the total renal lymph drainage

142 Tindall, A R (Institute of Biology and Geology University of Tromsø Norway) The activity of the rat ureter

The electrical and mechanical activities of the ureter have been recorded in situ by external recording devices Usually the electrical and mechanical activities are closely correlated progressing from the renal end of the ureter The frequency of contractions is about 25/min and the rate of propagation of the electrical activity is about 20mm/sec

The normal electromyogram probably arises in the first 5 mm of the renal end of the ureter and retrograde activity may arise from a similar region at the vesicular end of the ureter A close arterial injection technique shows that the proximal end of the ureter behaves as if it possess d alpha receptors while the distal end behaves as if it had beta receptors

Wallin, G., Delius, W [illegible]
ments of Clinical Neuro[illegible]
demic Hospital Uppsala [illegible]
PATHETIC OUTFLOW TO SKELETAL [illegible]

The microneurographic [illegible] muscle nerve sympathetic [illegible] types of heart arrythmias [illegible] arterial blood pressure [illegible] lay of 1 2 1 4 seconds was [illegible] mate correlation between the [illegible] occurrance of multiunit sym[illegible] corresponded to diastoles [illegible] during pulse intervals of lo[illegible] pressures The relationship be[illegible] interval (or the end diastoli[illegible] occurrance of the bursts was [illegible] shaped curve An approximately [illegible] between the duration of the [illegible] of the bursts The results [illegible] ting a strong baroreflex contr[illegible] the vascular bed of skeletal [illegible] plain previous findings of [illegible] vascular resistance in cases [illegible]

Blix A S H Refsum E L Gaut[illegible]

(Institute of Aviation Medi[illegible] Blindern Oslo Institute of [illegible] Zoophysiology University of [illegible] Hospital Oslo Norway) : [illegible]
VASOCONSTRICTION IN THE DIVI[illegible]

The effects of alpha-adre[illegible] parasympathetic (atropin) bl[illegible] sponse to diving in the do[illegible]

With no significant reduct[illegible] in heart rate alpha adrener[illegible] vasodilatation and virtuall[illegible] *of profound bradycardia dur*[illegible]

By both in vitro and in vi[illegible] fied that these effects coul[illegible] interference of phenoxyben[illegible] control

Atropinization eliminated [illegible] cardia while the arterial [illegible] creased suggesting that i[illegible] strictor fibres was maintai[illegible]

It is concluded that the [illegible] tion of the initial diving [illegible] <u>secondary</u> reflex adjustment[illegible]

145 Henriksen O & W P Paaske (Department of Nuclear Medicine Rigshospitalet Copenhagen Denmark & Institute of Medical Physiology B University of Copenhagen Denmark): INFLUENCE OF ORTHOSTATIC BLOOD PRESSURE CHANGES ON BLOOD FLOW IN SUBCUTANEOUS ADIPOSE TISSUE OF THE CRUS

The nature of autoregulation of blood flow in subcutaneous adipose tissue of the crus was investigated by means of the local xenon-133 washout technique Orthostatic blood pressure changes were obtained by passive displacement of the leg in relation to the heart

Blood flow remained constant from 20 cm below to 20 cm above the heart Outside this range blood flow decreased A decrement of blood flow with the leg in dependent position indicates an increase of vascular resistance as arterial and venous pressures increase in parallel below the heart

Patients suffering from intermittent claudication get notable relief of pain when they place their legs in dependent position This apparent increase of blood flow during dependency in these patients cannot be ascribed to an increase of the perfusion pressure head but rather by assuming that the vasculature in ischemic areas is paralyzed which might implicate a redistribution of blood flow from healthy areas with intact autoregulatory ability to ischemic regions creating an inverse Steal Syndrome

146 Eklund B and L Kaijser (Department of Clinical Physiology Karolinska Hospital Stockholm Sweden) CHANGES IN REACTIVITY OF THE MUSCLE VASCULAR u WITH THE DURATION OF EXERCISE

The I crease in muscle blood flow during work is considered due to in local chemical en ironment During prolonged work however lood flow is maintained in spite of a retu n towards resting levels of facto s such as PCO_2 osmolarity H^+ K^+ and lactate in the effluent venous blood To investigate if prolonged work changes the reactivity to vasoactive stimulation the effect on forearm blood flow of i a infusion of adrene gic asoactive substances such as norad enaline isoproterenol and phe tolamine and of sympathetic stimulation (lower body low pressure LBLP) was studied after 5 and 55 min dynamic forearm work Blood flow was measu ed with venous occlusion plethysmography

No adrenaline decreased flow both after short and prolonged work but I connection with prolonged work a pronounced increase was seen on termination of infusion which could be reduced by β adrenergic blockade It was not seen after LBLP Isoprote enol increased flow more after short than prolonged work Phentolamine increased flow markedly after prolonged but not afte sho t wo k Du ing work no adrenaline decreased SO_2 of the effluent enous blood more d ring prolonged than short work indicating a mo e p orounced flow eduction

It is concluded that prolonged work changes the vascular reactivity to such an e tent that it might be of impo tance fo flow regulation

Ljung, B and R Sivertsson (Depts of Physiology and Clinical Physiology II Univ of Göteborg Sweden): INHIBITION OF VASCULAR SMOOTH MUSCLE CONTRACTION BY VIBRATIONS 147

To elucidate whether vibrations in the cardiovascular system might exert a direct inhibitory action on smooth muscle contraction in blood vessels (e g post stenotic dilatation) we have characterized the influence of longitudinal sine-wave oscillations on contractile activity of isolated preparations of the rat portal vein and spiral strips of the rabbit thoracic aorta Low amplitude vibrations over a wide range of frequencies caused an almost instantaneous decrease in active force No decrease in passive tension was obtained when inactive tissues were vibrated The relationship between vibration rate amplitude and the relative inhibition was determined At a frequency of 200 Hz a 25 per cent decrease in active force of portal vein spontaneous activity and high K^+ contracture and of aortic noradrenaline and K^+ responses were obtained with vibration amplitudes of 2 1 8 and 6 per cent of tissue length respectively The results thus show that the oscillations cause prompt and pronounced inhibitions most likely due to a direct action on the contractile elements It seems probable that turbulence induced vibrations can influence vascular tone in some pathological conditions (arterial-stenosis; A-V-ana stomosis) and it is suggested that an inhibitory response to pulse wave oscillations might have a physiological significance in regulation of conduit blood vessel diameter (Supported by SMF 14X-3884)

Pyykkö I and J, Hyvärinen (Institute of Physiology University of Helsinki, Finland) STUDIES ON FINNISH LUMBERJACKS ABOUT THE PHYSIOLOGICAL MECHANISM THAT LEADS TO TRAUMATIC VASOSPASTIC DISEASE (TVD) 148

TVD is wide spread among Finnish chain saw operating lumberjacks In modern chain saws the dominant frequency of vibration is around 125 Hz In chain saws equipped with vibration dampers the peak-to-peak vibration amplitude of the rear handle is only 50–300 μ In this frequency and amplitude region the pacinian corpuscles are exquisitely sensitive to vibration. Vibration of a limb also causes discharge in abdominal sympathetic ganglia of the cat (Miyamoto, J and J Alanis Jap. J. Physiol. 1970 20 725) On the other hand, vibration of muscles leads to the tonic vibration reflex (TVR)

We recorded finger pulse volume, TVR, and galvanic skin response (GSR) in 43 chain saw operating lumberjacks with TVD Vasospastic reactions were provoked by body cooling and chain saw noise and particularly by vibration of hand At different vibration frequencies the threshold amplitude that produced vasospasms runs parallel and 20 dB above the threshold of pacinian corpuscles TVR and GSR appeared less directly related to the vasospasms Clonidine appeared to have a beneficial effect on the vasospasms in some patients

We suggest that TVD is produced through prolonged excessive activation of a sympathetic vasoconstrictor reflex triggered by activity in pacinian afferents

153 Ek L Dahlöf C Carlsson E Lisander B Martner J and Åblad, B, (Dept of Pharmacology AB Hässle Mölndal and Dept of Physiology University of Gothenburg Sweden): HEMODYNAMIC EFFECTS OF TWO ADRENERGIC BETA RECEPTOR ANTAGONISTS IN CONSCIOUS DOGS In the intact organism the acute cardiovascular effects of beta receptor blockade are probably mainly due to inhibition of adrenergic control of cardiac rate and contractility and to inhibition of the vasodilator effect of circulating adrenaline / Lately a new cardioselective beta receptor antagonist H 93/26 has been reported (Åblad et al Life Sci 12:107-119 1973) As regards blockade of the cardiac resp nses to cardiac sympathetic nerve stimulation H 93/26 was equipotent to propranolol while propranolol was a considerably more potent inhibitor of vasodilator effects of injected adrenaline than H 93/26 In anaesthetized cats and dogs the pressor respon e to adrenaline was potentiated by propranolol only / Because of these findings the two compounds may be anticipated to produce differentiated hemodynamic effects in physiological conditions where endogenous adrenaline is of importance for the cardio vascular control To study this question the acute cardiovascular effect of propranolol and H 93/26 have been compared in conscious dogs with chronically implanted devices under various conditions: at re t during exercise and in defence alarm situations It was found that during defence alarm situations the hemodynamic actions of the two beta receptor antagonist differed indicating a release of adrenaline

154 Nilsson S and D Grove (Department of Zoophysiology University of Göteborg Sweden and Marine Laboratories Menai Bridge Wales): AUTONOMIC NERVE CONTROL OF THE SPLEEN IN A FISH GADUS MORHUA

Measurements of flow through the perfused spleen at constant pressure show that splanchnic nerve stimulation acetylcholine noradrenaline adrenaline and phenylephrine but not isoprenaline increase resistance Quantitative studies show that cholinergic responses are selectively blocked by atropine and adrenergic responses by yohimbine or phentolamine Splanchnic nerve effects are rarely completely blocked by adrenergic or cholinergic antagonists alone Simultaneous adrenergic and cholinergic blockade regularly abolishes nerve responses Where reduced responses to splanchnic nerve stimulation were obtained following 6-hydroxydopamine or reserpine treatment these could be abolished by atropine alone Ganglion blocking agent (mecamylamine or hexamethonium) did not affect the responses to nerve stimulation

It is concluded that the spleen of Gadus is innervated by both cholinergic and adrenergic postganglionic constrictor fibres running in the splanchnic nerv

FAGRELL B (Departments of Medicine and Clinical Physiology Karolinska 155 institutet Seraflmerlasarettet Stockholm Sweden) VITAL CAPILLARY MICROSCOPY – A METHOD FOR EVALUATING THE EFFECT OF THEURAPEUTIC PROCEDURES ON ISCHEMIC SKIN ULCERS

It has been shown that structural changes of the skin capillaries – as studied by vital capillaroscopy (VC) – yield valuable information regarding the viability of the skin in patients with arterial vascular disorders thereby providing a means of estimating the potential risk for skin necrosis It has also been found that VC is of value for estimating the effect of therapeutic procedures on the nutritional skin circulation in patients with ischemic skin ulcers (Fagrell B Scand J clin Lab Invest Suppl 133, 1973)

In 1966 a new substance pyridinolcarbamate (PC) (a bradykinin antagonist) was reported to prevent vascular injury induced by atherogenic substances e g cholesterol (Shimamoto T et al Amer Heart J 71 216 1970 The present study was undertaken to evaluate the effect of PC on the structural appearance of the nutritional capillaries of the skin surrounding ischemic skin ulcers and to assess the possibility of using VC for predicting the prognosis of such ulcers

It was found that in 5 out of 6 patients with skin lesions where no or only a few capillaries were seen at the rim of the ulcers at the start of the treatment an almost normal number of blood-filled capillaries appeared after 8 weeks of treatment A marked improvement of the skin lesions was observed in these patients In the only patient where the capillary bed did not improved during the treatment the ulcer progressed successively

Paaske W P O Henriksen P Sejrsen & S Levin Nielsen 156 (Institute of Medical Physiology B University of Copenhagen Denmark): AUTOREGULATION OF BLOOD FLOW IN HUMAN CUTANEOUS AND ADIPOSE TISSUES

The presence of autoregulation of blood flow was investigated in subcutaneous adipose tissue of the distal forearm and in cutaneous tissue of the skin fold between thumb and forefinger by means of local xenon-133 washout techniques Orthostatic blood pressure changes were obtained by passive displacement of the area under study to various test levels above and below the reference level the jugular notch

Blood flow in both tissues remained constant for all subjects in an interval about the heart level By elevation of the arm out ide the autoregulatory range blood flow was found to decrease in proportion to the reduction in perfusion pressure By extreme downwards dislocation blood flow was suddenly reduced to about 60 per cent of the reference value Further lowering did not reduce blood flow any further

It is concluded that autoregulation of blood flow is present in cutaneous and adipose tissue in the interval from 5 cm below to 20 cm above the reference level as venous pressure is constant and arterial pressure is decreasing 20 mm Hg in this range

157 Bø G A Hauge and B A Waaler (Institute of Physiology University of Oslo Norway) THE SITE OF MECHANICALLY INDUCED CHANGES IN PULMONARY BLOOD VOLUME AND EFFECTS ON LUNG COMPLIANCE

Acute respiratory failure is frequently characterized by low lung airway compliance (C_L) increase in pulmonary blood volume (PBV) and interstitial edema Causal relationships are claimed to exist between these lung parameters Detailed mechanisms of interaction remain however to be elucidated

We have investigated the relative effects of lung edema and increases in PBV on C_L and also the effects of separate rises in arterial and venous pressures on PBV and C_L

In an isolated perfused and ventilated rabbit lung preparation lung weight and dynamic lung compliance were continuously recorded A rapid 50% rise in PBV induced by elevation of left atrial pressure (P_{LA}) at constant flow caused a simultaneous 30% fall in C_L With maintained high vascular pressure interstitial edema accumulated with no further fall in C_L When inflow pressure was elevated to the same levels 1) by a rise in flow at constant P_{LA} and 2) by a rise in P_{LA} at constant flow the latter type of experiment gave an increase in PBV 3 to 5 times larger than the first one The fall in C_L was closely related to the rise in PBV regardless of how such a rise was obtained The major part of the lung vascular capacitance changes took place in venous vessels

58 Ahrén, K Janson, P O, and Selstam, G (Department of Physiology and Department of Obstetrics and Gynecology University of Göteborg Sweden): PASSAGE OF MICROSPHERES IN FOLLICULAR AND LUTEAL RABBIT OVARIES.

Little is known about vascular mechanisms in the ovary during different endocrine conditions Regarding the ovarian blood flow it has been reported in the literature direct measurement methods yield about ten times higher values than indirect methods In order to test whether these differences could be due to arteriovenous anastomoses the passage of 15 ± 5 μ non-radioactive microspheres was investigated using _in situ_ perfusion techniques In the present study virgin Swedish Land rabbits, 5-6 months old were used. Ovulation was induced by intravenous injection of human chorionic gonadotrophin.

In one group of animals the passage of microspheres across the ovary was quantified by perfusing both ovaries _in situ_ at 75 mm Hg with a 6 % dextran solution holding a known concentration of microspheres All aortic branches except for the ovarian artery were tied off as well as the vascular connections to the uterus and the oviduct. The ovarian veins were cannulated and the venous effluent collected. The concentration of microspheres in the venous effluent was determined in a Burker chamber In another group of animals microspheres were injected into the aortic blood stream and the venous blood from the ovary was microscopically examined.

The percentage of microspheres passed in the first group was in both follicular and in early and late luteal ovaries around 1 %. All microspheres found in the venous effluent were 15 μ or less in diameter In the second group no microspheres were found in the venous effluent.

The results indicate that arterio-venous shunts alone in these types of ovaries cannot explain the above mentioned differences in blood flow rates obtained by the different measurement methods

Krarup N and J A Larsen (Institute of Physiology University of Aarhus Denmark) THE FUNCTIONAL IMPORTANCE OF HEPATIC ARTERIAL BLOOD FLOW IN CATS 159

Increased oxygen extraction from hepatic arterial blood may compensat for the reduction in portal blood flow seen in hemorrhagic shock in cats (Krarup N Acta physiol scand 1972) The aim of the present study was to demonstrate to what extent a reduction in hepatic art rial blood flow may be compensated by increased oxygen extraction from portal venous blood.

In 8 splenectomized cats the h patic artery was ligated after a control period and the following changes were observed portal venous flow increased 15% together with a slight decrease in portal venous pressure In 7 cats hepati venous oxygen saturation decreased from 49 to 23% and hepatic oxygen consumption was unchanged Liver function as determined from th splanchnic elimination of ethanol hepatic uptake and excretion of Indocyanine Green and bile flow was not affected In 1 cat in which the control oxygen consumption of the liver wa 60% higher than normal ligation caused an almost compl te (92%) desaturation of the hepatic venous blood accompanied by a reduction in hepatic oxygen uptake and liver function and an increase in hepatic 1 ctat output

It is concluded that when the hepatic oxygen consumption and portal blood flow are within normal limits a complete arrest of the h patic art rial blood flow may be compens ted by an incre se in portal venous oxygen extraction

Holmgren S and S Nilsson (Department of Zoophysiology University of Goteborg Sweden): DRUG EFFECTS ON ISOLATED FISH ARTERIES 160

Investigations of the effects of drugs on major arteries from a number of lower vertebrates led Burnstock (Pharmacol Rev 1969 21 247) to conclude that the vasomotor innervation in fish is primarily cholinergic whilst adrenergic control increased during evolution toward higher vertebrates Adrenergic nerves in fish arteries have been demonstrated (Kirby S and G Burnstock Comp Biochem Physi 1 1969 28 307)

In the present study the effect of cholinergic and adrenergic drugs on isolated strips from the celiac artery of the rainbow trout (Salmo gairdneri) and the cod (Gadus morhua) have been investigated

Rainbow trout art ri s are contracted by both noradrenaline (pD_2=8 02) and acetylcholine (pD_2=6 62) whil the cod arteries are sensitive t noradrenaline (pD_2=6 2.) but almost insensitive to acetylcholine (pD_2 less than 2 0)

The responses of the cod arterie to catecholamines suggest the presence of adrenergic α-receptors since the order of potency of the catecholamines is adrenaline > noradrenaline > phenyl phrine and isoprenaline does not produce any contraction. Furthermore the α-adrenergic blocking agent phentolamine and yohimbine competetively block the ffect f noradrenaline on the preparation.

It is concluded that in the cod as compared to the rainbow trout cholinergic receptors and thus perhaps cholinergic innervation play little rol in regulating the muscular tonus f the celiac artery

161 Koefoed-Johnsen, V I Lyon and H.H Ussing (Institute of Biological Chemistry A University of Copenhagen, Denmark): EFFECT OF CU ION ON PERMEABILITY PROPERTIES OF ISOLATED FROG SKIN (RANA TEMPORARIA)

Cu^{++} added to the external bathing solution of frog skin to a final conc of 10^{-5}M causes a marked decrease in chloride permeability and an increase in potential difference (Koefoed-Johnsen V and H H Ussing, Acta physiol scand 1958 42, 298) Chloride permeability decreases on an average to 1/4 of control values in frogs which have been kept at room temp for a day or more We have now found that the effect of Cu^{++} is much less pronounced in skins from cold stored (at 5°C) frogs Cu^{++} increase the s c c in cold adapted frogs an effect rarely seen in the warm adapted frogs

Sucrose and SO_4^{--} permeabilities are not affected by Cu^{++} treatment whether the frogs have been adapted to room temperature or not

The findings that sucrose and SO_4^{--} permeabilities are not affected by Cu^{++} while chloride permeability is considerably reduced seem to indicate two pathways for chloride ions 1) an intercellular shunt path which is not affected by Cu^{++} and 2) a cellular pathway which can be highly affected and reduced by Cu^{++}

162 Lang, L , Sjöberg E and C R Skoglund (Department of Physiology Karolinska Institutet, Stockholm Sweden): FROG SKIN GLAND ACTIVATION BY NERVE STIMULATION

Subcutaneous application of neurohormones has been a common way of activating frog skin glands whereas the effects of nerve stimulation have been less studied

In the present analysis a nerve-skin preparation from the calf, containing predominantly mucous glands was mounted so that the outside of the skin formed the bottom (0 63 cm^2 area) of a small test compartment of 0 5 ml volume filled with distilled water while the corium side made contact with Ringer solution in a lower compartment.

Glandular secretion was observed to occur at a stimulus strength of 3-5 V using pulses of 3 ms duration at 10 Hz; the conduction speed of the sympathetic fibers involved was found to be 2-4 m/s

Flame photometric determination of Na^+ and potentiometric analysis of Cl^- showed that optimal nerve stimulation at 10 Hz caused an increased outflow of Na^+ and Cl^- ions in equal amounts at a rate of about 1-2 ug NaCl per cm^2 skin area per 10 s

The concomitant conductance changes occurring in the fluid of the test chamber were continuously monitored by using a technique previously described (Haapanen L and C R Skoglund Acta physiol scand 1967 69 51) This proved to be a convenient method of getting approximative information of the quantities and time relations of the glandular secretions in the course of an experiment thus allowing e g direct comparison with skin potential changes

Frederiksen, O. (Inst for Experimental Medicine, University of Copenhagen, Denmark): EFFECT OF AMILORIDE ON THE TRANSEPITHELIAL FLUID TRANSFER MECHANISM IN RABBIT GALL BLADDER IN VITRO 163

Amiloride (AM), a potent specific inhibitor of transcellular Na transport in hypertonic transporting epithelia like toad bladder, blocks Na-uptake and transfer completely within 1 min when added to the mucosal side at concentrations of 10^{-7}-10^{-4}M It has been claimed (Crabbé & Ehrlich, Pflügers Arch. 304:284, 1968) that AM is without effect on Na-transport in small intestine

In the present study the effect of AM on transcellular fluid transfer in the isosmotic transporting rabbit gall bladder (GB) was studied 'in vitro' using a gravimetrical method 0 88 10^{-4} M was without effect 0 88 10^{-3}M produced a 60% reversible inhibition of mucosal-to-serosal net fluid transfer when applied from the mucosal side It was without effect from the serosal side Thus isosmotic transfer by the GB is sensitive to AM but to a smaller extent than hypertonic transporting epithelia Ca^{2+} removal from the mucosal side did not significantly alter the maximal response This is contrary to findings in the toad bladder (Cuthbert & Wong, Molec.Pharmacol. 8: 222, 1972) Preliminary results indicate decreased O_2-consumption with AM Furthermore, AM does not seem to alter total transepithelial resistance These findings suggest that the characteristics of ion uptake and / or transcellular ion transport are different in the two epithelial types

Nielsen, Robert (Institut of Biological Chemistry A, University of Copenhagen, Denmark): EFFECT OF THE POLYENE ANTIBIOTIC FILIPIN ON THE ACTIVE SODIUM TRANSPORT ACROSS THE ISOLATED FROG SKIN 164

It has been shown previously that addition of the polyene antibiotic amphotericin B to the medium bathing the outside of the frog skin caused an initial small increase in the short-circuit current (SCC) and the trans-epithelial potential (PD) followed by a great decrease in the PD (Nielsen R Acta physiol. scand 1971 83 106) Addition of filipin to the outside caused qualitatively the same effects as amphotericin B but the observed decrease in the PD was enhanced The addition of amphotericin B (5×10^{-5} M) to the inside had no effect on the PD and the SCC However the addition of filipin (5×10^{-5} M) to the inside caused an increase in SCC and PD This increase of the SCC starts after about 5 min of incubation with filipin Theactivation has its first maximum after 20-45 min of incubation thereafter the SCC starts to decline to about the control level After this decrease the SCC increases again and reaches its second maximum after about 2 hrs of incubation The increase in SCC varied from 10-100% of the control level

It is concluded that filipin added to the inside of the frog skin reacts at a site different from that it reacts with when it is added from the outside

165 Sjöström M   Johansson R   and Thornell L E   (Dept of Anatomy, University of Umeå Sweden): THE STUDY OF PHYSIOLOGICAL ION FLUXES BY MICROANALYSIS OF ULTRATHIN SECTIONS OF FROZEN TISSUE

In ultrathin sections of untreated tissue prepared by cryo ultramicrotomy naturally occuring ions can be identified *in situ* by means of electron microscopical X-ray microanalysis However in order for the data to be meaningful it is essential to define the functional state of the tissue at the time of freezing

Between two pneumatically controlled chilled hammers is an oxygenated bath In it a muscle is mounted between a micrometer screw and a tension transducer The bath contains two parallel electrode plates for stimulation of the muscle With the muscle stimulated or at rest the hammers are instantaneously brought together and at the same time the bath is drawn away The muscle is frozen between the hammers The muscle is in contact with air and thus unstimulated for less than 10 msec After dry-sectioning the sections are freeze-dried

Ultrastructure is well preserved which facilitates topographic orientation during analytical electron microscopy As muscular processes are instantaneously interrupted in a defined state of contraction and as diffusable substances are assumed to remain *in situ* during the preparative procedure it should be possible to study physiological fluxes of ions on the ultra structural level e g the flux of calcium during the contraction cycle X-ray analyses of sections are in progress

166 Ängo R and Åkerlund C (Department of Zoophysiology University of Göteborg Sweden) : EFFECT OF ESERINE ON FLUID BALANCE IN MOLLUSCS

Certain drugs have been observed to disturb water balance in lluscs Eggbert and Umrath (Z vergl Physiol 1956 39 133) noticed at eserine causes swelling of freshwater gastropods The mechanism the effect is not known We have investigated the effect of eserine and a few other drugs on a marine gastropod the Whelk (*Buccinum undatum*) The molluscs were placed in bowls with sea water at 11 °C The drugs were dissolved in the sea water or in a few cases injected Treated animals and controls were weighed at intervals At the end of the experiments the approximate blood volume was estimated by bleeding When whelks are treated with eserine or neostigmine at 10^{-6} – 10^{-5} gm/ml the animals after 2–3 hours are highly swollen The weight increase may be as high as 150 per cent of the initial body weight Measurements of the blood volume show that eserine induces an enormous increase of the blood volume Analyses of the blood showed no significant change of inorganic ions (sodium potassium calcium) but a marked decrease in plasma proteins The osmotic pressure did not change significantly The swelling was inhibited by sucrose at a conc of 300 mM/kg in the surrounding sea water The swelling seems to be secondary to muscle relaxation caused by the drugs

Schäfer, Almut and H - H Frey (Dept of Pharmacol and Toxicol School of Vet Med Free Univ Berlin-West Germany): EFFECTS OF PROSTAGLANDINS E_2 AND $F_{2\alpha}$ ON BRONCHIAL TONE IN CATS 167

The study should provide dose-effect relationships for the 2 PGs and disclose possible interactions with the autonomic nervous system Experiments were done in cats anesthetized with chloralose vagotomized completely relaxed by infusion of suxamethonium and artificially ventilated Bronchoconstriction was induced and maintained by infusion of 5-HT carbachol or histamine Against 5-HT PGE_2 in the dose-range of 0 05-10 µg/kg had a bronchodilator effect in all but 4 out of 22 cats in which the effect was constrictory PGE_2 was less effective against carbachol-induced bronchoconstriction and ineffective against histamine Dilator effects were slightly weakened by propranolol which in cats with normal bronchial tone intensified the constrictor effect Reserpine pretreatment seemed to increase the sensitivity to the dilator effect; results with guanethidine and cocaine were variable and chlorisondamine and phentolamine did not modify the dilator effect of PGE_2 This effect was dose-dependently intensified by indomethacin (2-10 mg/kg) - $PGF_{2\alpha}$ showed a rather weak bronchoconstrictor effect in the dose range of 0 1 50 ug/kg which could not be modified by any of the above mentioned drugs - The effects of the PGs on bronchial tone seem thus to be relatively independent of the autonomic nervous system but inhibition of PG synthetase by indomethacin resulted in an increased bronchodilator effect of PGE_2

Fjalland, B. (Department of Pharmacology and Toxicology H Lundbeck & Co A/S Copenhagen Denmark): INHIBITION BY ANTI-INFLAMMATORY AGENTS OF THE RELEASE OF RABBIT AORTA CONTRACTING SUBSTANCE (RCS) AND PROSTAGLANDINS (PGs) FROM CHOPPED GUINEA-PIG LUNGS 168

It has been shown that PGs and RCS are released from lungs and other tissues by anaphylaxis and by mechanical agitation (Palmer et al Br J Pharmac 1970 40 581P Gryglewski and Vane Br J Pharmac 1972 45 37) In the present study the effect of some anti-inflammatory agents on the mechanically indu ed relea e of PGs and RCS from chopped unsensitized guinea-pig lungs ha been investigated

Stirring of the lung ti sue resulted in relea e of activity corresponding to that of PGE_2 15 ng/ml as tested on the rat stomach strip The PG-release was proportional to the time of mechanical agitation of the tissu

Indom thacin when superfused over the lung tissue in a concentration of 20 ng/ml inhibited the release of PGs and RCS by about 50 % (IC50) The IC50 for aspirin was about 2 µg/ml Phenylbutazone was about 3 times as active as aspirin while odium salicylate wa inactive in concentrations up to 63 µg/ml

The results suggest that inhibition of mechanically induced release of pro taglandins may be u ed as a simple in-vitro te t for screening of anti-inflammatory agents

169 Gustafsson L P Hedqvist and H Lagercrantz(Dept of Physiology Karolinska Institute Stockholm Sweden): PROSTAGLANDIN MEDIATED ENHANCEMENT OF EFFECTOR RESPONSE TO CHOLINERGIC NERVE STIMULATION

Prostaglandin E_1 (PGE_1) has been shown to inhibit parasympathetic neuroeffector transmission in the rabbit heart(Wennmalm and Hedqvist Life Sci 1971 10 I 465) The present paper describes some PG actions in another cholinergically innervated tissue the bovine iris sphincter muscle

Strips of the muscle were mounted in an organ bath with oxygenated Tyrode s solution at 37°C Recordings of mechanical response were made isotonically Field stimulation(1 msec 1-3/sec 5 sec/min) was shown to activate cholinergic nerves causing contraction of the muscle

PGE_1 (0 2-1 0 ng/ml) potentiated contractions induced by transmural stimulation in a dose dependent manner Higher doses increased resting base line and caused direct contraction PGE_2 was equiactive with E_1 while $PGF_{2\alpha}$ was less effective in causing potentiation

Administration of the PG synthesis inhibitor Eicosatetraenoic acid (ETA) markedly to completely depressed the response to transmural stimulation.Addition of a small dose of PGE restored the response ETA also *removed the spontaneous and progressive increase in tone of teh* preparation normally terminating an experiment in 1-2 hours

Prostaglandins are released from the bovine iris sphincter muscle (Posner J Brit J of Pharmacol vol 40 no 1 163P-164P) We suggest that endogenous prostaglandins may modulate smooth muscle response to parasympathetic nerve stimulation

170 Stjärne, L, (Dpt of Physiology Karolinska Institutet Stockholm Sweden): MICHAELIS-MENTEN KINETICS OF CALCIUM-DEPENDENCE OF SYMPATHETIC NEUROTRANSMITTER SECRETION AND OF PROSTAGLANDIN DEPENDENT -INDEPENDENT FEEDBACK CONTROL OF THIS FUNCTION

In the isolated guinea-pig vas deferens the probability for (quant-) secretion of noradrenaline (NA) from sympathetic nerves on rival of th nerve impulse seems to be restricted by two different ntrol systems one dependent and one independent of the local release of prostaglandin E (PGE) The two systems seem to be additive in effect Both are sensitive to changes in the NA concentration of tissue fluid; the NA level required to depress transmitter secretion by 50% is about 100 ng/ml in the absence of PGE (local formation blocked by ETA) and about 50 ng/ml when PGE synthesis is not blocked The efficiency of both systems is inversely related to nerve stimulation frequ ncy Both mechanisms may operate by regulating the availability of calcium for the secretory m chanism Application of Michaelis-Menten kinetic analysis seems to allow determination of K_m (5 2 mM calcium) and V_{max} (4 2×10^{-5} of the total store released per nerve stimulus) for the secretory mechanism Exogenous PGE_2 (uncompetitively?) overcomes the disinhibition of NA secretion caused by alfa blockade; PGE_2 not only depresses V_{max} but also raises K_m progressively more with falling calcium concentration in the medium The potency of PGE suggests that the PGE-dependent control of NA secretion may operate a positive feedback loop (via (prejunctional?) alfa-adrenoceptors triggered by rising NA in tissue fluid) to depress the affinity for calcium of the s cretory mechanism

Hanje H Walum E and Edström A (Dept of Zoophysiology Göteborg Sweden): DIBUTYRYL CYCLIC AMP AND PROSTAGLANDIN E_1 INDUCE MORPHOLOGICAL ALTERATIONS IN CULTURED HUMAN GLIOMA CELLS 171

Neuroblastoma cells (C 1300) treated with dibutyryl cyclic AMP (dBo-AMP) or prostaglandin E_1 (PGE_1) differentiate within 24 h Protein- but not RNA-synthesis is necessary for this differentiation which fails if the integrity of the microtubular system is destructed (Prasad K and Vernadakis A Exp Cell Res 1972 70 27 ; Prasad K Nature New Biol 1972 236 49) Hamster ovary cells (CHO-K1) treated with the same drugs rapidly (1 h) change their morphology and become density dependent inhibited This effect is independent of protein- as well as RNA-synthesis (Hsie A and Puck T Proc natl acad sci 1971 68 358)

Cultured glioma cells (138 MG) normally have an irregular flattened shape We report here that 138 MG like C 1300 and CHO-K1 cells undergo morpological changes in the pres nce of dBo-AMP and PGE Within one hour the glioma cells elaborate up to 150 µ long multiple processes The alterations were accentuated in serum-free medium Morphological changes did not take place in the presence of vinblastine whereas they were unaffected by actinomycin D (10 µg/ml) as well as cyclo-heximide (40 µg/ml)

The vinblastine effect suggest that microtubuli are involved in the morphological changes of glioma cells The dBo-AMP and PGE induced changes appeared more rapidly showed reversibility and were in contrast to the effects on C 1300 not dependent on de novo protein-synthesis In the latter respects 138 MG resemble CHO-K1 cells

Eliasson R. Lindholmer C & Johnsen Ø (Reproductive Physiology Unit Dept Physiology Karolinska institutet S-104 01 Stockholm Sweden): EFFECTS OF HUMAN SEMINAL PLASMA ON SOME FUNCTIONAL PROPERTIES OF THE HUMAN SPERMATOZOA. 172

The seminal plasma has a remarkably complex composition indicating functions beyond that of providing nutriment for the spermatozoa during their short contact with the plasma and a vehicl for the transportation of spermatozoa into the female genital tract

Various functional properties of human spermatozoa have now been tudied in 1) whol semen 2) various fractions of "split-ejaculates (the first part of the human ejaculate contains mainly pro t tic fluid the last portion mainly fluid from the eminal vesicles) and 3) buffered salt solutions

The oxygen consumption of the spermatozoa was ignificantly higher in salt solutions than in whole semen The vesicular fluid had neg tive effects on sperm motility and survival and caused an incr ased uptake of zinc by the spermatozoa The prostatic fluid had a motility promoting ff ct on washed spermatozoa and protected the spermatozoa from the adverse actions exerted by the vesicular fluid

A better understanding of the functional relationship between the mal acces ory genit l glands and the spermatozoa i of importance from diff rent points of views Identification of functional parameters important fo the fertility of human spermatozoa and knowledge how such paramet rs can be influenced by endogenous and exogenous factors secreted by the accessory genital glands can give leads t new reversibl male contraceptive agents that does not interfere with the spermatogenesis

173 Persson N A and Hedqvist, P (Department of Physiology I Karolinska Institute Stockholm Sweden): REDUCED INTESTINAL MUSCULAR RESPONSE TO ADRENERGIC NERVE STIMULATION AFTER THE ADMINISTRATION OF PROSTAGLANDINS

Prostaglandins E (PGEs) inhibit sympathetic neuroeffector transmission in organs such as the spleen heart and vas deferens This paper describes similar effects produced by PGE_1 and PGE_2 on effector responses to adrenergic nerve activity in the rabbit intestine

Pieces of rabbit jejunum and ileum with intact adrenergic nerve supply were mounted in an organ bath and kept in oxygenated Tyrode at 37°C The adrenergic nerves were stimulated with square wave pulses (3 10 Hz 1 msec duration supramaximal voltage) and all recordings of mechanical activity were made isotonically (load 1 2 g)

In the absence of treatment the preparation showed spontaneous and rhythmic contractions Stimulation of the adrenergic nerves or administration of NA caused a graded inhibition of the intestinal movements Low doses of PGE_1 and PGE_2 (1 12 ng/ml) which per se showed little or no effect on the spontaneous contractions of the preparation consistently antagonized the inhibitory action of adrenergic nerve stimulation on intestinal motility in a dose-dependent manner On the other hand the inhibitory action of NA was little affected by the PGEs It is therefore suggested that the PGEs inhibit adrenergic responses in the intestine by reducing the release of NA from the adrenergic nerve terminals

174 USE OF CELL CULTURE TO STUDY BIOCHEMICAL, PHYSIOLOGICAL AND PHARMACOLOGICAL PROPERTIES OF ISOLATED NEURONS AND GLIAL CELLS L. Hertz and L. Dittmann

- of Biochemistry A University of Copenhagen DK-2100 Copenhagen and M Sensenbrenner and P Mandel Centre de Neurochimie du CNRS Strasbourg France

- Determination of metabolic events in the different cell types of the brain is encumbered with considerable uncertainty due to the complexity of the tissue Cell cultivation provides means either to obtain glial cells and neurons separately and without mutual contamination in amounts which are large enough (50-100 cells) to determine respiration by aid of Cartesian microdivers or to obtain cultures consisting of respectively pure glial cells and a mixed population of neurons and glial cells These cultures can be compared in fluxstudies The rate of oxygen uptake by the cultivated glial cell is relatively high (130 µmoles/g wet wt /h) though not as high as that by the neurons (570 µmoles/g wet wt /h) These values compare favorably with the respiratory activity in brain slices and in the brain in vivo An increase of the potassium concentration causes a significant rise of the oxygen consumption in the glial cells but has no effect on the neurons (cf Hertz J Neurochem. 1966 13 1373) Flux measurements (Latzkovits Sensenbrenner & Mandel in preparation) indicate a glial-neuronal interrelationship with respect to potassium transport Taken together the results emphasize the role of glial cells in brain physiology and biochemistry Pharmacological studies of the two cell types are in progress

S en A Andersson and Per E Loisaner (Department of Physio 175
logy University of Göteborg Sweden):
THE THALAMIC REPRESENTATION FOR SPINAL VENTRAL AFFERENT PATHWAYS IN KITTEN

In 197 we could report that activity evoked by electrical and adequate stimuli and m diated via ventral spinal afferent pathways will give a cortical response in young kittens most marked at the age of one to three weeks and after this age decreasing in size We have now examined the thalamic representation for this pathway in 30 kittens using microelectrode technique and identifying the recording site by physiological as well as histological methods

Sho t lat ncy potentials wor most evident in the region of nucleus centrum medianum ometimes with a high degree of con ergen e There were no re pon es from ventral pathways btained in VPL However some potentials with longer latency w re found anterior and posterior to this nucleus

Teyler T (+) and Skrede, K K (Institute of Neurophysiology University of 176
Oslo Norway) PRESERVATION OF SYNAPTIC MECHANISMS IN ISOLATED SLICES OF MAMMALIAN HIPPOCAMPAL CORTEX

A central question in the use of the slice technique regards the possibility of electrophysiological abnormality due to tissue damage

To study whether slices can show physiological responses comparable to those in intact preparations we used lices cut transversely to the longitudinal axis of the hippocampus in which the major pathways can be preserved due to its lamellar organization (Andersen Bliss & Skrede Exp Brain Res 1971 13 222-238) Such preparation contain a four-membered neuronal loop with three well-defined excitatory synapses (Skrede & Westgaard Brain Res 1971 35 589-593) The present study was intended to investigate synaptic facilitation and inhibition in on link of thi loop the connection between th CA3 and CA1 pyramids

Stimulation of these fibres and th alveus respectively gave orthodromic and antidromic population potentials of CA1 pyramids imilar to those *in vivo* The CA1 respons to repetitive orthodromic activation was a pronounced frequency-dependent facilitation Its time cours was examined using doubl bock techniques and found to be comparabl to that of th i tact preparation The phenomenon could be elicited without population pikes being recorded from any part of th CA1 area suggesting a non-recurrent mechanism A powerful inhibitory mechanism was also demonstrated which in contrast proved to be recurrent

We conclude that th de cribed inhibition and facilitation is of the same nature as demonstrated in *in vivo*

(+) Presently at Psychology Dept Harvard Univ Cambridge Mass U.S A

177 Hoff r B Ungerstedt U and Siggins G (Dept of Histology Karolinska Institutet Stockholm Sweden): ELECTROPHYSIOLOGICAL AND PHARMACOLOGICAL STUDIES ON CAUDATE NUCLEUS NEURONS AFTER DESTRUCTION OF DOPAMINE CONTAINING AFFERENTS

The effects of destruction of dopamine-containing afferents on the phy iological and pharmacological properties of caudate neurons was studied in halothane-anesthetized rats Action potentials from single neurons were recorded with multibarrel micropipettes and drugs were administered at the site of recording using microiontophoresis One week prior to recording 6-hydroxydopamine was injected unilaterally into the substantia nigra to liminate the dopamine projection to the ipsilat ral caudate In normal caudate the discharge of neurons is reproducibly and uniformly reduced by local application of dopamine or apomorphine After destruction of dopamine -containing affe ents by 6-hydroxydopamine the mean threshold iontophoretic curr nt for dopamine nd apomorphine inhibition are ignificantly reduced Moreov r the spontaneous di charge of caudate neurons i markedly elevated after elimination of dopamine input Normal caudate neurons usually discharge spontaneously at 1 ss than 0 5/sec or are silent After 6-hydroxydopamine most caudate units fire at rates of 2 5-5/sec; a significant proportion discharge at rates greater than 10/sec These results suggest (1) that apomorphine mimicks the effects of dopamin on caudate neurons (2) that apomorphine-induced inhibition is not mediated via dopamine -containing t rminals (3) that super nsitivity to the electrophysiological effects of dopamine and apomorphin develops after destruction of dopamine-containing afferents (4) that th dopamine pathway exerts a tonic inhibitory influence on caudate neurons

178 PLASTICITY OF CENTRAL NORADRENALINE (NA) NEURONS FOLLOWING ADMINISTRATION OF 6 HYDROXYDOPAMINE (6-OH DA) TO NEW BORN RATS <u>Chris Pycock</u> Charlotte Sachs and Gösta Jonsson Dept. of ~gy Karolinska Institutet Stockholm.

~mic injection of 6-OH DA (3x100 mg/kg s c.) to newborn rats was to cause a selective and permanent reduction in endogenous NA and A uptake in the forebrain being about 30% of control when analysed ~he adult stage However the NA concentration and ^3H NA uptake in the pons medulla region was increased by 75% Analysi of ^3H NA uptake kinetics showed similar Km values for 6-OH DA treated and control rats Subcellular distribution studies on endogenous NA in the pons medulla showed the highest relative NA increase in the microsomal fraction con sidered to contain the amine sto age granules The distribution pattern of NA synaptosome from the pons medulla loaded with ^3H NA and centri fuged on a ontinuous sucrose gradient differed between control and 6-OH DA t eated the peaks b ing at a sucrose concentration of 1 1 M and 1 25 M respectively Fluorescence histochemistry displayed a reduced number of nerve t rminals in the ce ebral cort x whereas in the pons medulla an inc eased numb r of nerve terminals with an increased fluor escence intensity wa seen. The present results show that 6-OH DA ad ministered at birth results in a selective degeneration of NA nerve ter minals in the fo eb ain whil in th pons medulla this treatment leads to an incr ase in NA concent ation in the nerve terminals and possibly also a growth of NA te minals p obably relat d to the damage of th terminals in the foreb ain

Skaugen, E. (Departments of Physics and Physiology University of Oslo Norway) POSSIBLE FUNCTIONAL SIGNIFICANCE OF THE GEOMETRY OF THE INITIAL SEGMENT IN NERVE CELLS 179

The density of Na^+ conducting pores in nerve membranes is rather low (Colquhoun D et al J.Physiol 227 95) The opening and shutting of the pores is a random process and the membrane potential will therefore tend to fluctuate The fluctuations may be noticeable and important in regions of nerve cells where the diameter and thus the area of the nerve membrane is small

Simulation of nerve cells with a uniform distribution of pores with equal properties has been performed on a digital computer The random process was governed by equations of the Hodkin Huxley type It was found that the action potential started where the axon had the smallest diameter (at the initial segment) When this diameter was decreased the firing frequency increased The relative increase was largest at small values of the injected current

It is concluded that a small diameter of the initial segment faciliates the initiation of the action potential at this place and makes the firing frequency a more linear function of the injected current

Røed, A (Institute of Physiology and Biochemistry, Dental Faculty University of Oslo, Norway) HIGH FREQUENCY INHIBITION OF THE SARCOLEMMA BY PROPRANOLOL 180

Propranolol has been shown to produce a high frequency inhibition of the rat phrenic nerve-diaphragm preparation during direct stimulation Twitch contractions were unaffected (Lilleheil G and Røed A Arch int Pharmacodyn 1971 194 129) In the present experiments the mode of action of propranolol on the sarcolemma was studied

Propranolol, $2x10^{-6}$g/ml did not affect low frequency muscle action potentials During 50/sec stimulation the action potentials appeared as an initial train with gradual development of initiation delay reduced steepness of rising and falling phases and amplitude reduction The train disappeared and was followed by irregularly appearing action potentials Greater inhibition was characterized by a shorter and less distorted train and reduced or abolished irregular activity Trains never reappeared during continued stimulation but could again be evoked after a short interuption of stimulation Accordingly the effect of propranolol is caused by an interference with stimulus excitation coupling and not by a prolonged refractory period An elevation of threshold was probably involved since the inhibition could be reduced by increasing the stimulating current

181 Leppäluoto J, Lybeck, H and Virkkunen P (Institute of Physiology, University of Helsinki Finland) INHIBITION BY HUMAN PLASMA OF THE BIOLOGICAL ACTIVITY OF THYROTROPHIN RELEASING HORMONE (TRH)

It has been shown that the biological activity of TRH is destroyed when the hormone is incubated with plasma Inactivation is believed to occur by splitting a part from TRH molecule or by binding TRH with plasma proteins (Bassiri R and Utiger, R Endocrinology 1972 91 657) We have studied the binding of TRH to plasma proteins and the capacity of various plasma fractions to inactive TRH

TRH (Ferring Ltd) was added to plasma submitted to gel filtration (Sephadex G-200) The plasma protein fractions were bioassayed for TRH in mice pretreated with T-3 and I 131 and they showed no TRH-activity Untreated plasma was then separated into 4 fractions by gel filtration and the fractions were incubated with TRH for 90 min TRH was in activated by the fraction with the relative elution volume of 1 6 - 1 8 We conclude that TRH is inactivated by a factor in plasma, possibly a protein enzyme

182 HELGASON S ST (Department of Zoophysiology University of Göteborg Sweden, and Department of Physiology, University of Iceland): EFFECTS OF CALCITONIN ON INDUCED HYPERCALCEMIA IN RAINBOW-TROUT *SALMO GAIRDNERI*

Parathyroid glands are absent in fish but ultimobranchial lls secrete calcitonin In some Teleosts calcitonin ases the calcemia in others, i e Salmonids calcitonin been reported to be without effect on plasma calcium

Most fishes are hypercalcemic with regard to their natural environment be it fresh water or sea water In the present experiment a severe calcium load was inflicted on 19 rainbow-trouts by adding $CaCl_2$ to fresh water to final concentration of 1 g/100 ml Another group of 10 fish was kept in fresh water Both groups received 40 mU calcitonin/100 g, and the calcemia was measured at t 0 and t 6 hours after calcitonin administration The fresh water group showed no changes in plasma calcium 6 hours after the injection Half of the fish kept in calcium rich water for one week had become strongly hypercalcemic and calcitonin injection decreased plasma calcium to normal values in 6 hours The remaining 5 fish, kept in calcium rich water, did not exhibit hypercalcemia after 7 days and calcitonin injection was without effect on plasma calcium

It is suggested that endogenous secretion of calcitonin in calcium treated fish which did not respond to exogenous calcitonin is sufficient to prevent hypercalcemia to develop

CHAN D K O *et al* *Gen comp Endocr* 1958 *11* 243-245
PANG P K T, *J Exp Zool* 1971 *178* 89-100

Russell J T and Thorn N A (Institute of Medical Physiology C University of Copenhagen, Denmark): EFFECTS OF INHIBITORS OF CALCIUM TRANSPORT (D600 AND PRENYLAMINE) ON RELEASE OF VASOPRESSIN IN VITRO 183

The presence of Ca^{++} is obligatory for the release of vasopressin from neurohypophyses in vitro and calcium seems to be taken up during the stimulation We have examined the effect on vasopressin release of D600 (a Verapamil derivative) and prenylamine which both inhibit calcium transport in smooth and cardiac muscle Halved rat neurohypophyses were incubated in a bicarbonate buffered medium with 2 8 mM Ca^{++} Stimulation of secretion was carried out by the application of a field current or with 56 mM K^+ containing medium D600 (4 10, 20 µM) and prenylamine (20 and 100 µM) produced a dose related inhibition of vasopressin release in both stimulation types D600 also inhibited the increased release caused by the introduction of Ca^{++} into a Ca^{++} free medium during electrical stimulation However prenylamine did not inhibit this release

The results suggest that an enhanced Ca^{++} transport over the cell membrane (which can be blocked by D600) precedes hormone release and that prenylamine acts at a subsequent step in the stimulation secretion coupling

Vilhardt H & Hope, D. B. (Department of Pharmacology University of Oxford, U K): DEMONSTRATION ON POLYACRYLAMIDE GELS OF ATPases FROM BOVINE NEUROHYPOPHYSES 184

Previous investigations (Poisner A M & Douglas W W, Mol Pharmacol 1968 4 531; Vilhardt H & Holmer G Acta endocr (Kbh) 1972 71 638) have demonstrated ATPase activity in homogenates and subcellular fractions of mammalian posterior pituitary glands

In the present experiments the ATPase activity of subcellular fractions of bovine neural lobes was studied. Three types of activity were found: Mg^{++}-, Ca^{++}- and Mg^{++} + Na^+ + K^+ activated The ratio between the 3 enzymes varied between the subcellular fractions The enzymes were all membrane-bound but the Mg^{++}- and Ca^{++}-ATPase could be solubilized by 1 % Triton X-100 with only minor loss of activity The activity of the Mg^{++} + Na^+ + K^+-ATPase was completely inhibited by this concentration of Triton X-100

The Triton X-100 solubilized proteins were subjected to electrophoresis on 5 5 % polyacrylamide gels The position of each ATPase in the gels was demonstrated by incubating the gels with ATP and either Mg^{++} or Ca^{++} followed by the addition of Pb^{++} The P_i liberated from ATP by the enzymes was precipitated as white bands in the gels In this way it could be shown that while the Mg^{++}-ATPase was also activated by Ca^{++} the Ca^{++}-ATPase seemed not affected by Mg^{++}

185 Møller, M K Møllgård & S C Sørensen (Anatomy Department A & Institute of Medical Physiology Dept A University of Copenhagen Denmark): CHANGES IN THE ULTRASTRUCTURE OF THE CAROTID BODIES FOLLOWING PROLONGED EXPOSURE TO HYPOXIA

The carotid bodies are considered primarily to be chemoreceptors in the ventilatory reflex response to changes in arterial pO_2 pCO_2 and pH but their ultrastructure suggests that they might be organs of internal secretion This study was performed in order to find out if their ultrastructure is changed following prolonged exposure to hypoxia Four rabbits were obtained in La Paz Bolivia (altitude 3800 m) They were bred and had lived at an altitude of 4000-4300 m Another four rabbits were exposed to hypoxia equivalent to an altitude of 6000 m in a low pressure chamber for seven days The carotid bodies were fixed by perfusion in vivo with glutaraldehyde postfixed with OsO_4 uranyl bloc-stained and embedded in Epon (R) In both groups we found a marked increase in the number of electron dense core vesicles and mitochondria in the type I cells compared to sea level controls

This finding is compatible with an increased production of an at the present time unknown substance in the type I cells during prolonged hypoxia

'reland L Kinemuchi H and Yoo B Y (Department of Phar- 1 , University of Umeå Sweden): THE MECHANISM OF ACTION THE MONOAMINE OXIDASE INHIBITOR PARGYLINE

Pig liver monoamine oxidase has previously been shown to contain one mole of covalently bound FAD per mole of enzyme It has also been shown that by inhibition with the irreversible inhibitor N-methyl-N-2-(propynyl)-benzylamine (Pargyline) the inhibitor is bound to the enzyme in an equimolar ratio to flavin In order to investigate the nature of the binding of Pargyline to the active site purified pig liver monoamine oxidase was inhibited by ^{14}C-labelled Pargyline and then extensively digested by pronase The Pargyline-containing fragment was purified by gel filtration ionic exchange chromatography and thin layer chromatography The equimolar ratio between Pargyline and flavin was shown to be retained after the purification procedure Thin-layer chromatography in several systems with subsequent localization of flavin in UV-light and Pargyline by autoradiography has further indicated that Pargyline is bound to the flavo-peptide Amino acid analyses of the Pargyline-containing flavo-peptide after performic acid oxidation yielded cysteic acid aspartic acid serine and glycine in a molar ratio of 1:1:1:2

Hansen A J & S C Sørensen (Institute of Medical Physiology Dept A University of Copenhagen Denmark) CHANGES IN THE LDH ISOENZYME PATTERN IN RABBIT BRAINS FOLLOWING PROLONGED HYPOXIA 187

In most tissues there are five LDH-isoenzymes each composed of different proportions of H and M subunits It has been shown in various tissues in vivo and in vitro that the relative concentration of H-subunits decreases when P_{O_2} is lowered This is assumed to reflect a greater anaerobic glycolysis in the tissue We have compared the LDH-isoenzyme pattern in cortical grey matter corpus callosum cerebellar cortex and inferior colliculi from rabbits exposed to hypoxia equivalent to an altitude of 6000 m for seven days with that in rabbits living at sea level The LDH-isoenzymes in supernatants from homogenates were fractionated by Agarose gel electrophoresis and the relative concentrations of H subunits were calculated from the scanning curves The concentration of H subunits (mean sem) in six hypoxic rabbits and six sea level rabbits respectively were: cortical grey matter: 71 7±0 8 and 70 5±1 3; corpus callosum: 74 0±1 7 and 76 0±1 4; cerebellar cortex: 81 2±0 9 and 73 5±1 2; inferior colliculi: 89 5±0 8 and 81 5±0 6 The differences are only significant in cerebellar cortex and inferior colliculi ($p<0$ 001) Thus there is regional differences in the changes in the LDH-isoenzyme pattern However the results suggest that in some areas of the brain the degree of anaerobic glycolysis is increased during prolonged hypoxia which might be of importance for the ventilatory acclimatization to chronic hypoxia

Oreland, L and Kinemuchi H (Department of Pharmacology University of Umeå Sweden): THE SUBUNIT STRUCTURE OF PIG LIVER MITOCHONDRIAL MONOAMINE OXIDASE (MAO) 188

The molecular weight of pig liver MAO has previously been shown to be about 115 000 with one mole of covalently bound FAD per mole of enzyme Gel filtration of purified enzyme on Sepharos 4 B in 6 M guanidine and 0 1 M mercaptoethanol (MCE) and analytical ultracentrifugation in 0 1 % sodium dodecyl sulphate (SDS) and 0 1 % MCE yielded molecular weights of 55 000 and 63 000 respectively By polyacrylamide gel electrophoresis in 0 1 % SDS + MCE one band of 62 000 mwt appeared Those results seems to imply that the enzyme is composed of two subunits of which one carries the active site If MCE was omitted during the gel electrophoresis two equally large bands of about 60 000 mwt were formed By using enzyme inhibited by ^{14}C-Pargyline a MAO-inhibitor blocking the active site of the enzyme in a 1 1 molar ratio it was found however that both bands contained Pargyline in a ratio of 1 mole per 140 000 g of protein Furthermore amino acid analyses yielded tho same amino acid composition of the two bands The results are interpreted that the enzyme is composed of two subunits of identical molecular weight (about 60 000) of which only one contains the active site and that the enzyme preparation contained two forms of the enzyme presumably differing in the state of oxidation of SH-groups

189 Herlitz, H. and R. Hultborn (Department of Physiology and Institute of Neurobiology University of Göteborg, Sweden): ADVANCES IN A SPECTROPHOTOMETRIC PROCEDURE FOR MEASUREMENTS OF OXYGEN CONSUMPTION IN MICRO-SCALE.

A microspectrophotometric procedure for determination of respiration of minute tissue samples has been developed as an alternative to existing micromanometric techniques (Herlitz H. and Hultborn, R. Acta physiol. scand 1973 87 4A; Hultborn, R. Anal. Biochem 1972 47 442) The tissue sample is incubated together with an oxyhemoglobin solution within an air-tight glass-chamber and the absorbance change of the solution is recorded spectrophotometrically when the oxyhemoglobin is deoxygenised. The sensitivity can be varied from 10^{-6} - 1 µl O_2/hr by changing the volume of the incubating chamber In the present study samples of rat corpus luteum tissue were assayed for oxygen consumption in absence and presence of succinate using chambers drilled in object slides giving a volume of approx. 1 µl and a sensitivity in the order of 10^{-2} µl O_2/hr This system has been characterized by our group (Herlitz, H. and Hultborn, R. Acta physiol. scand. 1973 87 4A) and the methodological results can thus easily be evaluated. Three major improvements have been introduced since the former report (Herlitz, H. and Hultborn, R. Acta physiol. scand. 1973 87 4A): 1 An automatic cuvet changing device has been developed increasing the measuring capacity 6-fold enabling up to 24 determinations a day 2 Reagents can be introduced into the chamber during the experiment so that metabolic effects can be studied using the same sample of tissue thus minimizing biological and experimental variation. 3 O_2-affinity of hemoglobin is decreased using inositol hexaphosphate thus increasing the oxygen tension at which recordings of respiration are performed.

190 Edman K.A P and M Johannsson (Department of Pharmacology University of S-223 62 Lund Sweden) THE CONTRACTILE STATE OF RABBIT PAPILLARY F IN RELATION TO STIMULATION FREQUENCY

There is evidence (Koch Jaser and Blinks Pharmacol Rev 1963 15 601) the cardiac action potential in addition to being a trigger of the mechanical activity exerts both a positive and a negative effect on the subsequent contractile state of the muscle The aim of the present study has been to further elucidate these two effects Rabbit papillary muscles (37 °C) were stimulated by passing rectangular DC pulses (2 ms duration) through platinum plate electrodes The stimulation frequency was varied between 0 3 and 420 per min AC stimulation (20 Hz) was also used The conventional force-frequency curve was determined from measurements of the steady-state twitch amplitude at different stimulation rates The curve exhibited a maximum at 200 300 stimuli per min The twitch amplitude in response to a test stimulus applied at different times after a stimulation period was studied It was deduced from such measurements that the negative effect of excitation disappeared with a time constant of 150 ms whereas the positive effect of excitation declined with a time constant of approx 100 s The twitch amplitude recorded 1 s after a preceding stimulation period was used as an index of the maximal contractile state of the muscle (MCS) at the stimulation frequency considered MCS increased steadily with the stimulation frequency The highest value of MCS was recorded after a period of 20 Hz AC stimulation this value being 2 5 times greater than the steady state twitch tension recorded at optimal frequency An increase in the external calcium concentration from 2 mM to 8 mM had little effect on the peak value of MCS

Harri M,N E, R Tirri and L, Laitinen (Zoophysiological Laboratory, Department of Zoology, University of Turku, Finland): LOWERED CHRONOTROPIC SENSITIVITY OF RAT AND FROG HEARTS TO SYMPATHOMIMETIC AMINES FOLLOWING COLD ACCLIMATION 191

Cold acclimation is known to increase the metabolic sensitivity to sympathomimetic amines in mammals (Carlson, L D, Pharmac, Rev 1966 18 490) as well as in amphibians (Harri, M, and R Hedenstam, Comp Biochem Physiol 1972 41A 409) Thus, the effect of cold acclimation on the chronotropic sensitivity of the heart was studied

The test drugs were injected intravenously to anesthetized rats and to pithed frogs Rana temporaria

- Cold acclimation elevated the heart rate in the rat and decreased the chronotropic sensitivity to noradrenaline and isoprenaline These changes were temporary Phenylephrine failed to increase the heart rate In the frog, cold acclimation lowered the chronotropic sensitivity of the heart most to phenylephrine, less to adrenaline and not at all to isoprenaline, as indicated by changes in the ED50-values In addition, the elevation of the measuring temperature decreased the sensitivity to phenylephrine in this animal

The results obtained are suggested to be explained by decreased receptor sensitivity and increased metabolic rate and COMT activity in the heart tissue caused by increased sympathetic activity at low temperatures

Kaijse, L L A Carlson, B W Lass rs H Löw M Wahlqvist (Department of Clinical Physiology and Endocrinology and King Gustaf V Research Insti tue Karolinska sjukhuset Stockholm and Department of Geriatrics Uppsa la University Sweden): SUBSTRATE AND HORMONE CONCENTRATIONS IN THE RE GULATION OF SUBSTRATE UPTAKE BY THE HUMAN HEART 19

The myocardial extraction of a substrate is mainly determined by the blood concentr tions of substrates and hormones We have studied the relative importance for the extraction of a substrate of its own blood concentration concentrations of other substr tes and hormones | In normal men by coronary sinus catheterisation both in the fasting state after lowering plasma free fatty acid (FFA) concentrations by nicotinic acid and increasing substrate concentrations by the infusion of glucose and a fat emulsion

Myocardial extractions of glucose FFA and lactate were correlated with their arte ial concentrations Glucos extraction was not directly cor elated with ins li concentration but multiple regression analysis revealed a significant corr lation with i suli as well as growth hormone glucoco ticoid nd FFA A suming only one independent variabl was altered at a time a 10 % increase in rterial concentration of either insulin growth hormone glucocorticoid or FFA would result in respectively 25 % increase 6 % decrease 11 % decrease or 10 % decrease in myoca dial glucose extraction Exogenous f t in the form of chylomicrons seemed to exert the same n gative effect on glucose extraction as did FFA

193 Ilebekk A and Lekven J (Institute for Experimental Medical Research University of Oslo Ullevål Hospital Norway) EFFECTS OF NICOTINE ON CARDIAC MECHANICS

Nicotine increases ventricular stroke volume (SV) but it is not known whether this effect is a consequence of increased end-diastolic volume or increased myocardial shortening during the ejection period To examine this problem ventricular dimensions and fiber shortening (MS) were measured by ultrasound technique (Bugge-Asperheim et al. Scand J clin Lab Invest 1969 24, 361) on anesthetized open-chest dogs

Intravenous infusion of nicotine (20 µg/kg min) raised SV by $25 \pm 7\%$ and left ventricular systolic pressure (LVSP) by 37 ± 6 mm Hg Both MS and end-diastolic dimensions rose ($p < 0\ 01$)

When LVSP was raised by aortic constriction to the same value as during nicotine infusion SV was slightly reduced Myocardial contractility and MS were also slightly reduced but end-diastolic dimensions increased more than after nicotine ($p < 0\ 05$)

It is concluded that increments in both MS and end-diastolic ventricular dimensions contribute to the increase in SV Since end-diastolic dimension rose more during aortic constriction the increased MS represent the predominating cardiac mechanical event leading to increased SV under the influence of nicotine

194 Haglund U O Isaksson and O Lundgren (Dept of Physiology University of Göteborg Sweden) : CARDIAC EFFECTS OF BLOOD BORNE MATERIAL RELEASED FROM THE SMALL INTESTINE IN SHOCK

A pronounced cardiovascular derangement is often observed in the cat after a two hour period of a simulated shock situation in the small intestine (regional hy- at 30 mm Hg during activation of the nervous vasoconstrictor fibres) It proposed that these effects were caused by blood borne cardio-depressant substance(s) released from the shocked" small intestine (Haglund and Lundgren, Acta physiol scand in press) In order to obtain further evidence for this hypothesis a study on an in vitro heart preparation has been performed Rat hearts were perfused anterogradely according to the technique of Morgan et al (Fed Proc 1965 24 1040) By this technique both the aorta and left atrium are cannulated and the heart is performing an actual mechanical work 40 ml of oxygenated Krebs Henseleit bicarbonate buffer containing glucose was recirculated and the left ventricular pressure was continuously monitored by a pressure transducer After an initial perfusion period to stabilize the heart function 2-4 ml of venous plasma from control or shocked intestinal segments was added to the perfusion medium In 9 of 10 experiments shock plasma induced a pronounced depression of cardiac function within 2-8 min as revealed by a decrease in peak systolic pressure Control plasma caused no such effects Thus the present results strongly suggest that cardio-depressant material is released from the small intestine in shock Moreover this heart preparation seems to be a convenient bioassay system in evaluating what kind of substance(s) that induce the cardiac hypofunction in shock

Galbo H and P E Paulev (Institute of Medical Physiology B University of Copenhagen Denmark) CARDIAC ECHO DIFFERENTIATION (CED) DURING REST AND SUPINE CYCLING 195

Using pulsed reflected ultrasound the displacement (s(t)) of an easily recognized area of the posterior left ventricular wall can be recorded On line differentiators were developed to differentiate the s(t) curves hereby providing the velocity ($\dot{V} = dS/dt$) and acceleration ($\ddot{A} = d^2S/dt^2$) of the epicardial movement observed The standardized technique was evaluated in six healthy young men with phonocardiography and stroke volume determinations (CO_2 rebreathing method) both at rest and during supine cycling (150-300-450-600-750-900 Kpm/min) The positive peak values of the first and second time derivatives of the s(t) curve were located almost constant relative to the auscultatory systole at all exercise levels and the period of positive velocity (CED-systole) constituted approximately 90% of the auscultatory systole being equivalent to the ejection period at least at rest The peak displacement (ΔS) increased linearly with stroke volume and adjustment for individual heart volume showed that stroke volume was proportional to the arbitrary variabl (ΔS heart volume). The four subjects with the largest maximal oxygen uptake per weight unit had the largest increment in positive peak velocity (V) per heart rate increment indicating that the fittest subjects had a higher contractility increment per unit of tensile stress The CED examination is practicable even during heavy exercise and might yield physiological information concerning alterations in stroke volume and myocardial contractility on a beat to beat basis

Edman K.A.P A. Mattiazzi and E Nilsson (Department of Pharmacology University of Lund S-223 62 Lund Sweden): TEMPERATURE DEPENDENCE OF THE FORCE-VELOCITY RELATIONSHIP IN CARDIAC MUSCLE 196

The force-velocity (P-V) relation in rabbit papillary muscle was analyzed at various temperatures (23-32 °C) using the damped-release method described previously (Edman and Nilsson Acta physiol scand 1972 85 488) A lowering of the temperature induced a considerable increase in P_o (the tension at zero velocity) whereas Vmax (the shortening velocity at zero load) was only slightly affected This is in contrast to the effects produced by various inotropic interventions (cardiac glycosides adrenaline, calcium and altered stimulation frequency) in which cases Po and Vmax are affected to very nearly the same degree The temperature-induced changes of the P-V curve were interpreted as being the net result of two opposing effects: 1 an altered degree of activation of the contractile system caused by a change in the concentration of activator-calcium at the contractile sites (inotropic temperature effect) and 2 a change in the rate of interaction between the A- and I-filaments (specific temperature effect) The latter effect would be reflected by the change in Vmax after correction for the inotropic effect For the purposes of the present analysis the change in Po was used as a measure of the inotropic temperature effect By allowing for an equally large inotropic change of Vmax (cf above) the specific temperature effect could be derived Using this approach the Q_{10} of the specific temperature effect was found to be 2 0 ± 0 3 (mean ± SEM n=8) This value agrees well with the temperature dependence of Vmax during tetanic contraction of skeletal musel (Close Physiol Rev 1972 52 129)

197 Lekven J (Institute for Experimental Medical Research University of Oslo Ullevål Hospital Norway) ENLARGEMENT OF ACUTE MYOCARDIAL ISCHEMIC INJURY BY DOPAMINE.

Adrenergic stimulation of the heart increases the myocardial oxygen requirements due to increased mechanical activity and excessive myocardial consumption of free fatty acids (FFA) The purpose of the present study was to test whether ischemic injury of the myocardium might be aggravated by dopamine treatment Comparison was made between the effects of dopamine (120 µg/min) and calcium (1 5 mEq/min) on myocardial ischemic injury following acute coronary artery occlusions in 12 anesthetized dogs Ischemic injury was measured as the sum of S-T segment elevations in 9-11 epicardial ECG recordings (ΣST)

ΣST averaged 26 ± 4 mV at control occlusions At reocclusions during inotropic stimulation ΣST was 73 ± 12 mV with dopamine but only 41 ± 7 mV with calcium ($P < 0\ 005$) although the ventricular performance was similarly raised by the two drugs Arterial concentrations of FFA rose by 258 ± 61% with dopamine but remained unchanged during calcium infusion

Similar experiments were made after inhibition of lipolysis with β-pyridyl carbinol (9 mg/min) No differences in ΣST or arterial FFA concentrations could be demonstrated during dopamine and calcium infusions

It is concluded that stimulation of lipolysis contributes significantly to the enlargement of acute myocardial ischemic injury caused by dopamine

198 Diamant B , S Norn P Felding N Olsen, A Ziebell & J Nissen (Dept of Pharmacology University of Copenhagen Denmark) ATP LEVEL AND CO_2 PRODUCTION OF MAST CELLS IN ANAPHYLAXIS

The influence of anaphylaxis on ATP level and oxidative metabolism was examined in mast cells from sensitized rats Mast cells were isolated (Fl 11) and resuspended in buffer (pH 7) including 1 mM Ca^{++} 1 mg/ml human albumin and 1 mM pyruvate as substrate After preincubation of the for 20 min at 37°C antigen (horse serum 0 7% final conc) or buffer controls) was added and the incubation was continued up to 2 hr ATP level o change in ATP was found with controls (10 15mol/mast cell) After anaphylaxis a significant decrease was obtained 25% after 2 min and 40% after 5 min with no further change up to 2 hr Similar results were obtained when pyruvate was excluded When 5 mM glucose was used as substrate no changes or differences were found between anaphylactic and control cells CO_2 development In the control cells the CO_2 production from C^{14} pyruvate (1 mM) was found to increase linearly with time ($2\ 3 \times 10^{-16}$mol CO_2/min/mast cell) After anaphylaxis a significant increase (approx 30%) was found up to 2 hr CO_2 production was completely inhibited by antimycin A (10^{-6}M) indicating that it represented oxidative metabolism. To examine whether an uncoupler of oxidative phosphorylation could mimic the effects of antigen the experiments were repeated with 10^{-4} and 10^{-5}M of 2 4 dinitrophenol instead of horse serum. Similar results were obtained To conclude Anaphylaxis caused a decrease in the ATP level of the mast cells and an increase in their CO_2 production which could be the consequence of an uncoupling effect of oxidative phosphorylation induced by the antigen-antibody reaction Grants Danish Med Res Council and Danish Found for the Advancement of Med Science

Peterson C. and Diamant, B (Dept of Pharmacology Karolinska Institutet Stockholm Sweden) UTILIZATION OF ENDOGENOUS ATP DURING HISTAMINE RELEASE FROM ISOLATED RAT MAST CELLS 199

Histamine release from mast cells induced by compound 48/80 is blocked by inhibitors of energy metabolism (Diamant and Uvnäs 1961) in contrast to when n-decylamine or chlorpromazine is used as releasing agent (Högberg and Uvnäs 1960 Frisk-Holmberg 1971)

After the addition of antimycin A (0 2 µM) to suspensions of mast cells the ATP content of the cells decreased to 1/3 of the original value in 2-3 min. Concomitantly the ability of the cells to release histamine when exposed to 48/80 decreased The addition of glucose (0 3-0 6 mM) to antimycin A treated cells partially restored ATP and concomitantly the ability to release histamine when exposed to 48/80 was restored

To investigate if the energy dependence of 48/80-induced histamine release reflected an increased energy utilization during the release process the ATP content of mast cells was measured before and after histamine release had occurred. When cells with an intact energy production were used the content of ATP was unchanged after the addition of 48/80 However when cells were treated with antimycin A for 1 min the decrease of the ATP content was significantly greater after histamine release had been induced by 48/80 than in control samples or when histamine release had been induced by chlorpromazine or n-decylamine

It is suggested that histamine release induced by 48/80 but not that induced by chlorpromazine or n-decylamine is accompained by an increased utilization of endogenous ATP and that cells with an intact energy metabolism immediately restore the ATP level

Johansen T and Chakravarty N (Dept of Pharmacology Odense University 5000 Odense Denmark) THE RELATION OF ADENOSINE TRIPHOSPHATE CONTENT OF RAT MAST CELLS TO ANAPHYLACTIC AND COMPOUND 48/80-INDUCED HISTAMINE RELEASE 200

Pure populations of rat peritoneal mast cells were isolated by differential centrifugation in concentrated serum albumin The cell suspensions in Krebs-Ringer solution were preincubated with varying concentrations of oligomycin to reduce the adenosine triphosphate (ATP) content of the mast cells Histamine release was induced thereafter either by antigen (egg albumin) using mast cells from actively sensitized rats or by the polymeric amine, compound 48/80 ATP was assayed by the bioluminiscence technique and histamine by the fluorometric method

Oligomycin gave similar but not identical dose response curves for the reduction of ATP content and the inhibition of histamine release In general the degree of reduction of the ATP content showed a good correlation with the inhibition of histamine release Eighty to 100 per cent reduction of the ATP content was associated with almost complete inhibition of the release

The observations are consistent with the view that energy requiring processes are involved in the anaphylactic histamine release from mast cells

201 Anderson, P S A Slorach and B Uvnäs (Department of Pharmacology, Karolinska Institutet Stockholm 60 Sweden) CORRELATION BETWEEN HISTAMINE RELEASE AND ULTRASTRUCTURAL CHANGES IN NORMAL AND SENSITIZED RAT MAST CELLS IN VITRO EVIDENCE FOR AN EXTRACELLULAR RELEASE OF HISTAMINE

Mixed cell suspensions were taken from peritoneum and pleura in normal and sensitized (egg-albumin and pertussis vaccine) male Sprague-Dawley rats The cells were incubated either with 0 4 µg/ml compound 48/80 at 17°C or 5 µg/ml egg albumin at 25°C (sensitized cells) Samples were taken for histamine assay and electron microscopy The latter were fixed in 2% glutaraldehyde and 1% OsO_4 dehydrated and embedded in Vestopal W or Araldite Lanthanum nitrate and hemoglobin were used in some experiments as extra cellular tracers Untreated mast cells or mast cells taken before the histamine release started at 10 sec or 30 sec (sensitized cells) showed the ultrastructural appearance of normal non-degranulating mast cells with homogeneous electron dense granules surrounded by perigranular membranes The lanthanum and hemoglobin precipitates were adsorbed to the cell membrane but none was found inside The initial changes were always found in the most peripherally - located granules Such granules showed a swollen appearance and a decreased electron density compared to normal granules All changed granules were shown to be in communication with the extracellular space Histamine release were correlated to the granule changes and increased to 65% after 3 min with compound 48/80 and 21% with antigen during this time the above changes affected more and more mast cells and penetrated more deeply into the mast cells

202 Sörenby, L (AB Draco Research and Development Laboratory Lund Sweden): THE INHIBITORY EFFECT OF TERBUTALINE ON THE ANTIGEN-INDUCED RELEASE OF HISTAMINE FROM THE GUINEA-PIG LUNG.

-pigs were sensitized with ovalbumin in Freund s complete adjuvant Two to four weeks later the lungs were challenged with ovalbumin in vitro The incubation fluids and boiled tissue extracts were assayed on the atropinized guinea-pig ileum The amplitudes of the rapid contractions were compared with those of known histamine solutions and here referred to as histamine

There was an increase of histamine in the incubation fluid and a concomitant decrease of tissue histamine after treatment with ovalbumin 0 01 1 0 mg/ml Terbutaline 2 10^{-8} - 2 10^{-6} M added immediately before ovalbumin counteracted the histamine release Propranolol 2 10^{-6}M and INPEA 2 10^{-5}M blocked the inhibitory effect of terbutaline The effective terbutaline doses are in accordance with those for dilatation of the guinea-pig trachea It is concluded that terbutaline counteracts the antigen-induced release of histamine from the guinea-pig lung The effect is mediated by β -receptor stimulation

Melander A F Sundler & U Westgren (Departments of Pharmacology and Histology, University of Lund Sweden): SIGNIFICANCE OF THYROID MAST CELLS IN THE REGULATION OF THYROID ACTIVITY 203

The significance of thyroid mast cells in the regulation of thyroid activity was studied in vivo and in vitro on rats mice and calves A combination of histochemical chemical and physiological procedures was used

Rodent thyroid mast cells were found to contain 5-hydroxytryptamine and histamine whereas the calf thyroid mast cells contained dopamine and histamine The number of thyroid mast cells could be correlated to the plasma TSH level and an increase in the plasma TSH level stimulated the formation of mast cells within but not outside the thyroid TSH also evoked a rapid release of amines from thyroid mast cells Release of mast cell contents induced by compound 48/80 was accompanied by increased secretion and synthesis of thyroid hormone and by an increased thyroid blood flow These effects could be mimicked by the amines TSH and compound 48/80 induced an increase in thyroid blood flow only when a significant number of mast cells was found in the thyroid

It seems probable that the formation of thyroid mast cells is controlled by TSH, and that the TSH-regulated activation of the thyroid is facilitated and partially mediated by amines that are released from thyroid mast cells by TSH

Lie M Johannesen J and Kiil F (Institute for Experimental Medical Research University of Oslo Ullevål Hospital Oslo Norway) EFFECT OF CYANIDE ON CORTICAL AND OUTER MEDULLARY METABOLIC RATES 204

Cyanide blocks active tubular Na reabsorption but it is controversial to what extent all segments of the tubules are affected (M. Martinez-Maldonado et al Am J Physiol 217:1363 1368 1969 S W Weinstein and R.M Klose Am J Physi l 217: 498-504 1969) To examine the distribution of the effects on renal metabolism after cyanide injection into the renal artery cortical and outer medullary metabolic rates were measured in anesthetized dogs using the heat accumulation technique GFR fell by 43 5 ± 3 2% and tubular Na reabsorption by 53 3 ± 3 5% after cyanide infusion at rates of 10 µmol/min. Metabolic rat was reduced in the outer medulla by 56 1 ± 2 3% in the cortex by 54 3 and 3 2% the diff rence not being statistically significant After distal blockade of sodium reabsorption with ethacryni acid and chlorothiazide cyanid had no further effect on outer medulla metabolic rate whilst cortical metabolic rate was reduced by 37 9 ± 3 4%

It is concluded that cyanid blocks active sodium reabsorption to an equal extent in proximal tubules and ascending limb of Henle s loop in contrast to the effects of ethacrynic acid and chlorothiazide mainly located in the distal nephron

205 Møller, O J (Institute of Physiology University of Aarhus Denmark): DOES THE BRUSH BORDER OF THE PROXIMAL TUBULE CONTAIN A (Na + K)-ACTIVATED ATPase

Whether potassium is transported actively or passively in the proximal tubule of rabbit kidney is a matter of dispute Because of the electrical and concentration gradients active transport (if it occurs) must take place across the luminal membrane The presence here of a (Na + K)-ATPase would strengthen the thesis of active K-transport at this interface

Brush border fragments of proximal tubule were prepared according to Thuneberg and Rostgaard (Exp cell res 51 (1968) 123) By electron microscopy they appeared to be almost uncontaminated by other membrane material Such preparations had a (Na + K)-activity of 0 6 µmoles Pi split/mg protein per min and an alkaline phosphatase a marker enzyme for brush border activity of 1 8 µmoles Pi split/mg protein per min

The preparations were treated by gradient centrifugation as well as by continuous free-flow electrophoresis but it did not prove possible to alter the ratio of (Na + K)-ATPase/alkaline phosphatase by more than two times Although it is likely that (Na + K)-ATPase is present in brush borders its presence only in contaminating (non brush border) membrane fragments could not be excluded

206 Sohtell M and H R Ulfendahl (Institute of Physiology and Medical Biophysics University of Uppsala Sweden) INTRACELLULAR CHLORIDE ACTIVITY OF THE PROXIMAL TUBULES IN THE RAT KIDNEY

...de reabsorption in the proximal tubules is generally accepted to be ... but there still remains the problem whether the transport route is ... or intercellular Several factors are of importance for transcellular ...loride transport among other the intracellular chloride activity The aim ...f this investigation was to study the intracellular chloride activity with liquid ion exchange electrodes

The electrode was of a glass open tip type with an outer diameter of less than 1 micron where the very tip was filled with a chloride selective liquid The reference electrode was an Ag/AgCl electrode with a KCl bridge and had the same capillary design The in vivo measurements were made on rats where the left kidney was reached via a flank incision and immobilized in a cup With micromanipulators the proximal tubular wall was punctured

The potential measured is the sum of the chloride and the transmembrane potential In another series the transmembrane potential had to be measured with Ling-Gerad electrodes As the potentials were generally transient and showed a large amplitude scattering the evaluation of intracellular chloride activities became very difficult The results indicate an intracellular chloride activity of 10-50 mM

Karlmark B Persson A E G Wistrand P, and B Ågerup (Dept of Physiology and Med Biophysics and Dept of Pharmacology Univ of Uppsala Sweden) 207

ON THE QUESTION OF AN INTRALUMINAL CARBONIC ANHYDRASE AS A FACTOR IN THE RENAL BICARBONATE REABSORPTION

The postulated mechanisms behind the bicarbonate reabsorption from the proximal tubule of the kidney include the combination of filtered bicarbonate ions and secreted hydrogen ions to form intraluminal carbonic acid This substance would rapidly decompose into water and carbon dioxide under the influence of carbonic anhydrase which is located on the luminal side of the tubular cell As carbon dioxide probably is the major substance in this chain which is reabsorbed the specific inhibition of this postulated enzyme ought to diminish the rate of bicarbonate disappearance from the tubular lumen

The experiments were performed on white rats with one kidney prepared for micropuncture studies Bicarbonate solutions were instilled into the proximal tubules using the Gertz technique The rate of pH decrease (rate of bicarbonate reabsorption) in the droplets was measured with an antimony micro electrode system Four different solutions were used all of them containing 150 mM $NaHCO_3$: a) pure bicarbonate b) bicarbonate + Acetazolamide c) bicarbonate + a dextran–coupled inhibitor and d) bicarbonate + a new polar inhibitor The carbonic anhydrase inhibitors in the two last solutions were designed to act exclusive intraluminally

The results show a markedly delayed acidification using the acetazolamide solution as compared to the others The rate of acidification using the intraluminal inhibitors was not different from that of the unmixed bicarbonate solution – Thus, the results do not show any evidence for an intraluminal carbonic anhydrase activity

SKINNER, S.L (Institute for Experimental Medicine University of Copenhagen Denmark) 208

: PHYSIOLOGICAL IMPLICATIONS OF RENIN KINETICS

To establish the influence of substrate concentration and co-factors on reaction velocity renin substrate was purified from plasma of nephrectomised sheep rabbits and rats and also from pregnant women Kinetic constants for the semi-purified homologous reactants were compared with those from unpurified plasma in 0 16M saline-phosphate buffer at pH 7 4 All systems contained 0 001 MEDTA and were angiotensinase-free. Initial velocity of angiotensin formation was determined by bio-assay in the ganglion-blocked rat. Sheep substrate was purified 1 200 times over normal plasma (8 mM/mg protein) in 2 steps on ion-exchange and gel-filtration chromatography with better than 80% recovery At this purity Km for the homologous sheep reactants (2µM) was identical with that found in unpurified whole plasma The homologous rabbit (Km = 2µM) and rat (Km = 2 5µM) and human reactions (Km greater than 3µM) could also not be established as different between purified and unpurified preparations

Maximum velocity for the human reaction was not reached even at substrate concentrations of 10µM (0 14µg/mg protein) Km could not be accurately determined but is predicted as considerably greater than 3µM. Km for the heterologous reaction human renin on sheep substrate was 0 29µM and readily determined In all of these species normal substrate concentration is at or near the first order range and exercises control over the 'in vitro' production rate of angiotensin No evidence for reaction co-factors was encountered Physiological studies of plasma renin concentration and substrate in rabbits and man indicated that first order kinetics also has an important influence on in vivo renin activity

209 Sheikh, M I (Institute of Medical Biochemistry University of Aarhus Denmark)

RENAL HANDLING OF PHENOL RED A COMPARATIVE STUDY ON THE ACCUMULATION OF PHENOL RED AND p-AMINOHIPPURATE IN RABBIT KIDNEY TUBULES <u>IN VITRO</u>

The characteristics of renal accumulation of phenol red and p-aminohippurate (PAH) have been compared It has been found that the aerobic accumulation of phenol red is about 4-5 times as high as that of PAH Furthermore phenol red is accumulated by renal tissue under anaerobic conditions in contrast to PAH The aerobic accumulation of phenol red as compared to PAH is less susceptible to inhibition by probenecid 2 4-dinitrophenol (DNP) and octanoate whereas fumarate and succinate affect the aerobic accumulation of both phenol red and PAH to the same degree Finally studies on renal homogenates showed that phenol red is bound to constituents of renal tissue in particular to the microsomal and mitochondrial fraction It is concluded probenecid DNP and octanoate cause more inhibition of organic anion transport than fumarate succinate and PAH because of lipophilic interaction with the membrane

210 Christensen S (Department of Pharmacology University of Copenhagen Denmark): RENAL CONCENTRATION ABILITY OF RATS WITH LITHIUM-INDUCED POLYURIA

[illegible] treated chronically with subtoxic doses of Li develope con-[illegible]ole polyuria It is uncertain to what extent the distur-[illegible] is due to primary polydipsia or to impaired renal concen-[illegible]ation ability To elucidate this problem, dehydration experi-[illegible] were carried out in (1) rats with polydipsia induced by replacing drinking water with 5% glucose, (2) rats with pituitary diabetes insipidus and (3) Li-polyuric rats Each group was deprived of water for 72 hours or until 20% loss of body weight Weight loss diuresis and urine osmolality were recorded every 6 hours and serum osmolality at the end of the deprivation period The initial urine osmolalities were 100-300 mosm/kg in all groups Maximum urine osmolalities during dehydration were (1) 2540-2880, (2) 970-1160 and (3) 960-1880 mosm/kg Terminal serum osmolalities were (1) 310±1, (2) 246±9 and (3) 382±34 (S D) mosm/kg In the Li-group (3) the maximum urine osmolalities were linearly correlated with the initial values It is concluded that lithium-polyuria in rats is due to decreased renal concentration ability rather than to a polydipsic action of Li

Skadhauge E & Bradshaw S D (Inst of Medical Physiol A Copenhagen and Dept of Zoology Univ of Western Australia): 211
SALT AND WATER EXCRETION IN THE AUSTRALIAN ZEBRA FINCH

Zebra Finches (ZF) from Western Australia were previously observed to drink saline up to 0 8 M whereas domesticated ZF only drank 0 3 M NaCl and excreted droppings of maximally 700 mOs ZF caught at Mileura 800 km NE of Perth (annual rainfall 5 cm) were studied Drinking of 0 8 M NaCl was confirmed but the average osmolality of the urine (supernatant of the lower end of the droppings after spinning at 26000 g) was only 1027 mOs The osmotic urine to plasma ratio was 2 79 identical to that in the dehydrated state (2 78) This indicates that ZF of arid zones most likely represent a genetically select subgroup

In order to investigate why these ZF can drink fluids more concentrated than the droppings the water turnover was measured in the dehydrated state The food intake was recorded and the droppings were collected quantitatively under oil The 24 hr water turnover (preformed and metabolic water) was estimated to 24% of the total body water This calculation was confirmed by measurement of the 24 hr turnover of HTO (23%) Drinking of 0 8 M NaCl seemed to occur since the intake only amounted to 10% of the water turnover in the dehydrated state It increased the average urine Na and Cl concentrations from 7 6 and 39 8 meq/l respectively in the dehydrated state to 117 and 161 meq/l This probably caused a greater water absorption in the cloaca

Weiss George B (Department of Pharmacology University of Texas Southwestern Medical School Dallas Texas USA): USE OF LANTHANUM TO DELINEATE MECHANISMS OF CALCIUM-DEPENDENT ACTIONS IN DIFFERENT ISOLATED MUSCLE SYSTEMS 212

In a numb r of smooth and striated muscle preparations La^{3+} can displace Ca^{2+} from surface (stabilizing) ites and inhibit the uptake of Ca^{2+} to less superficial sites or stores In some systems (especially guinea pig ileal longitudinal smooth muscle) La^{3+} may also alter the mobility of Ca^{2+} present at less superficial sites; this effect is an indirect one and exists as a function of the degree of interaction between uperficially-bound Ca^{2+} and Ca^{2+} located at less sup rficial sites Conversely the increased Ca^{2+} uptake elicited with either caffeine (in frog artorius muscl) r nicotine (in frog rectus abdominis muscle) is not blocked by La^{3+} Thus in each type of muscle employed it is ne essary to exclude the possibility that alterations in ^{45}Ca content may result in part from La^{3+}-resistant effects on uptake or efflux of ^{45}Ca. Exposure to La^{3+} as well as depletion f Ca^{2+} inhibited K^+-induced ontractions more than thos licited with norepinephrine or histamine in rabbit aortic trips with acetylcholine in rat uterus and with caffeine in frog sartorius muscle However La^{3+} inhibited only the initial portion of the response of frog rectus abdominis muscle to high K^+ nicotine and acetylcholine whereas Ca^{2+}-depletion completely blocked responses to all three of these agents

All of the specific effects observed with La^{3+} can be directly related to an interference with the manner in which cellular Ca^{2+} is taken up stored and utilized in each distinct type of muscle Use of La^{3+} in this manner can help identify mechanisms by which various pharmacological utilize Ca^{2+} in different types of smooth and triated muscle

213 Batra, S (Dept of Pharmacology University of Lund Sweden): EFFECTS OF CERTAIN DRUGS ON Ca UPTAKE AND Ca RELEASE BY MITOCHONDRIA AND SARCOPLASMIC RETICULUM OF FROG SKELETAL MUSCLE

The effects of quinidine chlorpromazine and caffeine on Ca uptake and Ca release by mitochondria and fragmented sarcoplasmic reticulum (FSR) were studied Quinidine (1-2 mM) released considerable Ca from preloaded mitochondria but had little effect on preloaded FSR The uptake of Ca both by mitochondria and FSR was inhibited by higher concentrations (2 or 1 mM) of quinidine but the inhibition of mitochondrial Ca uptake was much greater With lower concentration (0 4 mM) there was no significant effect on Ca uptake by FSR but a 48 % inhibition of mitochondrial Ca uptake was observed

Chlorpromazine (0 01-0 1 mM) inhibited Ca uptake by both mitochondria and FSR but the inhibition in the case of FSR was weaker than mitochondria Only the highest concentration (0 1 mM) of chlorpromazine caused a release of Ca from mitochondria or FSR Caffeine (2-10 mM) inhibited Ca uptake both by mitochondria and FSR and again the inhibition of Ca uptake by mitochondria was greater than of FSR Caffeine (10 mM) in contrast to quinidine released Ca from FSR and not from mitochondria

Ca releasing concentrations of these drugs were comparable to those reported to elicit contractures of living muscle Lower concentrations which inhibited Ca uptake were comparable to those which potentiate twitch

Haffner J F W B -I Jesheim & J Setekleiv(Institute of Pharmacology University of Oslo Norway):K-EFFLUX AND THE ADRENERGIC RESPONSE IN ISOLATED RABBIT STOMACH MUSCLE

- studies presented(Haffner J F W B-I Jeshein & J Setekleiv Acta pharmacol et toxicol 1972 31 412;1973 1974 in press) were carried out to determine whether the variable mechanical responses to adrenergic agents in rabbit stomach muscle were correlated with alterations in K-efflux Fundus and antrum strips were loaded with ^{42}K mounted in a perfusion chamber and washed with Krebs-solution Adrenaline noradrenaline phenylephrine or isoprenaline was added after an equilibration period of 28-32min Tension and K-efflux were recorded Phenylephrine noradrenaline and adrenaline produced significant (p o o5) increases in K-efflux both when the mechanical response was excitatory(increased tone in the fundus initiation of contractions in the antrum strips)and inhibitory(inhibition of contractions in antrum strips) and also when no mechanical response occurred Nor was any correlation found between the magnitude or time course of the mechanical response and the increase in K-efflux from fundus strips Isoprenaline had no effect on K-efflux

It is concluded that the increase in K-efflux produced by alfa-receptor stimulation appear to be of no causal importance for the mechanical response in rabbit stomach muscle

Masheim B I (Institute of Pharmacology University of Oslo Norway) ACTION OF NORADRENALINE AND ISOPRENALINE IN THE OESTROGEN AND THE PROGESTERONE DOMINATED RABBIT UTERUS 215

Hypogastric nerve stimulation contracts uteri from oestrogendominated rabbits and relaxes uteri from progesterone dominated rabbits It has been postulated that the adrenoceptors in the oestrogen dominated myometrium are predominantly excitatory (α-receptor) and in the progesterone dominated myometrium predominantly inhibitory (β-receptor) This hypothesis has been examined by in vitro stimulation with noradrenaline and isoprenaline of muscle strips taken longitudinally from the uterine horns The strips showed spontaneous activity and isoprenaline caused an inhibition of this activity There were no differences in the dose response curves from oestrogen and progesterone dominated uteri

Noradrenaline increased the frequency and the basal tone of the contractions to the same degree in oestrogen and progesterone dominated strips In oestrogen dominated strips the force of the contractions was increased up to three times the force of the spontaneous contractions In progesterone dominated strips the force of the contractions was not increased

It is concluded that the difference between the oestrogen and progesterone dominated uteri seen on sympathetic nerve stimulation depends on different reactions to α-receptor stimulation

Lagercrantz, H. (Dept. of Physiology I Karolinska Institute Stockholm Sweden): NORADRENALINE AND PROTEIN RELEASE FROM ISOLATED SYMPATHETIC NERVE VESICLES 216

Noradrenaline (NA) has been assumed to be released together with its storage components from sympathetic nerve v sicles by exocytosis in the same way as catecholamines (CA) are ecr ted from chromaffin granules (se Smith & Winkler Handbook of Experimental Pharmacology XXXIII:537 1972)

In vitro studies of highly purified nerve vesicles isolated from bovin splenic trunk have demonstrated significant differences between these vesicles and tho e isolated from th adrenal medulla: 1) While the chromaffin granule consists of about 80 % of a water soluble CA: ATP: protein complex; the nerve vesicle preparation was found to contain less than 20 % soluble proteins and dopamine β hydroxylase only trace amounts of chromogranin A and also l ss ATP (NA/ATP ratio = 9 4) 2) Incubation at 30° C resulted in a rapid spontan ous release of NA (48 % after 10 min) mainly ind pendent of protein and ATP release (only 2 % dopamine β hydroxylas was relea ed after 10 min) The chromaffin granul s releas CA much slower under similar conditions and there i a parallell loss of oluble p oteins and ATP (se Stjärne Handbook of Experimental Pharmacology XXXIII:231 1972)

Conclusion: Th nerve vesicle seem to sto e only one fifth of its NA in a water soluble complex with proteins and ATP Such a complex is assumed to be released by exocytosis

117 Serck-Hanssen Guldborg (Institute of Pharmacology, University of Oslo, Norway): SECRETION OF ADRENOMEDULLARY CATECHOLAMINES EFFECTS OF THEOPHYLLINE AND PROPRANOLOL

Methyl xanthines have been shown to stimulate the secretion of catecholamines from the adrenal medulla (Berkowitz B A and Spector, S Eur. J Pharmacol 1971 13 193) and to potentiate the secretion of catecholamines induced by cAMP (Peach, M J , Proc Nat Acad Sci 1972 69 834)

In the present work the secretion of catecholamines from retrogradely perfused bovine adrenals has been studied Acetylcholine in half maximum dose 10^{-4}M caused the release of adrenaline and noradrenaline in ratios of approximately 2 3 1 The presence of 1 mM theophylline in the perfusion medium (Tyrode s buffer) potentiated the effect of acetylcholine about 30 % and produced a relative increase in the amount of adrenaline released The effect of theophylline was also observed in glands blocked with 10^{-7}M atropine Propranolol in a concentration of 2×10^{-7}M at which it had no membrane stabilizing effect, reduced the acetylcholine-induced secretion about 20 %

The present results indicate that the acetylcholine-induced secretion of catecholamines from the adrenal medulla is partly mediated by a β- adrenergic receptor As the β-receptor is closely associated with the adenyl cyclase the secretion from the medulla seems partly to be controlled by cAMP

...ng, E (Institute of Pharmacology University of Oslo): STIMULATION OF THYMIDINE UPTAKE BY ACTINOMYCIN D IN HEPATOMA CELL CULTURE

Preincubation with actinomycin D for 2 hours increases the uptake of ^{3}H-thymidine into the acid-soluble fraction of HI_1C_1 cell cultures with a linear log dose-response curve from 0 01 μg/ml to 10 0 μg/ml Actinomycin D at a dose of 1 0 μg/ml gave 200-250 % stimulation; increase in the dose above that which completely blocked RNA synthesis still increased thymidine uptake A parallel increase in activity in the acid-insoluble fraction was found The effect was detectable after 5 minutes but was maximal first after 1-2 hours treatment with the drug This effect could not be blocked by cycloheximide Actinomycin D preincubation for 2 hours inhibited ^{14}C-hypoxanthine uptake into the acid-soluble fraction with a linear log dose-response curve from 0 01 μg/ml to 10 0 μg/ml; 50 % inhibition was seen at 0 75 μg/ml 10 0 μg/ml gave 73 % inhibition Actinomycin D preincubation altered the V_{max} of the thymidine and the hypoxanthine uptake but did not change the K_m s of the two reactions Actinomycin D did not change the activity of thymidine kinase in vitro

Garberg, L Borg K-O Olbe L Sjöstrand S E Sundell G 219
(Pharmacological dept AB HH ale Mölndal Sweden and Surgical Clinic II Sahlgrenska Hospital Gothonburg Sweden): EVALUATION IN THE DOG OF A NEW GASTRIC ACID SECRETION INHIBITOR

H 81/75 a potent inhibitor of gastric acid secretion in the rat (Sundell et al) has been studied in several experimental models in the dog

In dos s of 20-50 mg/kg i v H 81/75 was found to inhibit gastric acid secretion in dogs in which vagal activity has been stimulated However when pentagastrin or histamine were used as stimulators no effect was found General pharmacological studies have shown that the compound is not to be considered as a general anticholinergic agent

Pharmacokinetic studies using ^{3}H-labelled compound have been carried out in the dog A difference in the rate of elimination f H 81/75 in the dog and the rat was found

It is concluded that H 81/75 is a more limited inhibitor of gastric acid secretion in the dog than it is in the rat Data support a peripheral ite of action in the dog

Sundell G Borg K-O Garberg L Palmer L Sjöstrand S E 220
(Pharmacological dept AB Hässle Mölndal Sweden) EVALUATION OF A NEW GASTRIC ACID SECRETION INHIBITOR H 81/75 IN THE RAT

H 81/75 is a arbamate tructure: N-(2-methoxyethyl)-propylcarbamate Its effect on ga tric acid secretion in the rat ha been studied in different experimental models including the pylorus ligated rat (Shay rat) and the acute fistula rat both under basal conditions and on pentagastrin histamine or ₓ-DG timulated secretion

In the Shay rat ED_{50} for inhibition of gastric acid secretion was 1 5 mg/kg

In the acute fistula rat duration studi were performed H 81/75 show d more than 40 % inhibition of basal ga tric acid ecretion during 4 hour after administration of a single do e 6 mg/kg A do e-dependent inhibition of stimulat d ga tric acid secr tion was found

Pharmacokinetic studie have be n performed with ^{3}H-labelled compound in the acute fi tula rat The e showed a linear relationship between plasma concentration and antisecretory effe t on basal gastri acid s cretion

Thu H 81/75 was found to be a pot nt inhibitor of both spontane u and stimulated gastric acid ecretion in the rat

221 Thiringer G & Svedmyr Nils (Lung Clinic University of Göteborg Sweden) COMPARISON OF I V ADMINISTERED AND INHALED TERBUTALINE WITH DOSE EFFECT CURVES IN PATIENTS WITH CHRONIC OBSTRUCTIVE LUNG DISEASE

Tremor is the most usual side effect when giving the new relatively selective adrenergic $beta_2$ stimulators Tachycardia is the dose limiting effect only in few patients

We have recorded dose response curves for the effect of terbutaline given i v and by inhalation on the bronchi ($FEV_{1.0}$) heart rate blood pressure and muscle tremor Terbutaline given i.v to patients with chronic obstructive lung disease in doses that did not relax the bronchial muscle maximally more than doubled the tremor and increased the pulse rate about twenty beats/minute The tachycardia is mainly due to peri pheral vasodilatation and reflexogenic heart stimulation. When terbuta line was given by inhalation the same bronchial relaxation was obtained without any effect on circulation or muscle tremor indicating a local effect. The acute margin of safety was notable as the pulse increase after 63 inhalations of this long acting substance was only about twenty beats/minute Conclusion It was not possible to obtain maximal bronchodilatation with terbutaline given i v in patients with chronic obstructive lung disease without side effects When terbutaline was given by inhalation much better relaxation of the bronchial muscle could be reached before the side effects occurred

Pelkonen O. [illegible] E Larmi T K. I & Kärki N T (Departments Pharmacology and Surgery University of Oulu Oulu Finland):
LIVER DRUG-METABOLIZING ENZYMES IN DIFFERENT DISEASES

Clinical and experimental studies have demonstrated impairment of oxidative drug metabolism in diseases affecting liver (for ref see Aurenen A. Acta Chirurg Scand 1972 Suppl 424) Earlier we have studied in vitro activities of drug-metabolizing enzymes in liver biopsy samples from patients with cholelithiasis (CL) (Pelkonen O. et al Scand J Clin Lab Invest 1973 31 suppl 130 24)

In this study liver samples were obtained from patients with obstructive icterus (8), cirrhosis (7) and cancer (13) Samples were studied with respect to cytochrome P-450 and b5 NADPH-cytochrome c reductase 3,4-benzpyrene and aniline hydroxylases aminopyrine N-demethylase and hexobarbital oxidase Values obtained were compared with those of CL-patients (36)

Mean levels of drug-metabolizing enzymes were decreased about 10 - 40 % in obstructive icterus and 20 - 70 % in cancer patients (mostly stomach carcinoma with or without metastases) The impairment of drug metabolism increased when the histological appearance of the liver sample changed from normal to malignant. No significant decrease in enzymes was observed in patients with hepatic cirrhosis. Also no significant difference was detected between CL-livers with normal histological appearance and CL-livers with mild to moderate inflammatory and degenerative changes.

Mjörndal T and Oreland L (Department of Pharmacology University of Umeå Sweden): PLASMA LEVELS AND CLINICAL EFFECTS OF THIORIDAZINE AND THIOTHIXENE 223

The effects of thioridazine and thiothixene were studied by a double blind technique on 40 schizophrenic patients The doses were adjusted for optimal clinical effects and ratings for therapeutic and side-effects made after three and eight weeks of treatment No statistically significant differences were obtained between the two drugs or between any of the two drugs and the previous medication

Plasma levels were estimated by a fluorometric technique previously described by us after three and eight weeks of treatment No correlation was obtained between plasma levels and clinical effects for either thioridazine or thiothixene Between doses and plasma levels a clear correlation was found for both drugs after three weeks of treatment After eight weeks this correlation remained for thioridazine but not for thiothixene By that time serum levels of thiothixene had decreased about three times indicating strong enzyme induction

Sellman R Pekkarinen A & Raijola, E (Department of Pharmacology Turku University and Psychiatric Clinic of Turku Municipal Hospital Turku Finland) REDUCED PLASMA DIAZEPAM CONCENTRATIONS IN CHRONIC ALCOHOLIC PATIENTS FOLLOWING AN ORAL ABSORPTION TEST OF DIAZEPAM 224

Generic equivalence was observed in an earlier oral absorption study of three diazepam preparations (Kangas et al Annals Clin Research 1973 to be published) Diazepam (D) (Tensopam) 10 mg was given orally to 12 chronic alcoholics (I) most of whom were heavy drinkers at the beginning of the alcohol free period and to 14 controls (II) D was determined gas chromatographically with a ^{63}Ni EC detector During the absorption phase of D at 2 and 3 5 h following administration of the tablet the blood levels of D were reduced by 43 % ($p<0$ 01) and 32 % ($p<0$ 001) respectively in I compared with II (140 $\pm$ 13 and 88 $\pm$ 12 ng/ml resp) Seven subjects in I showed very low values of D during the absorption phase (<45 ng/ml) Neuroleptic drugs (chlorprothixene perphenazine or chlorpromazine) were given to 9 of the subjects in I but the earlier study has shown that this treatment does not alter the absorption profile of D compared with that seen in controls Two of the 5 alcoholics who had taken dichloralphenazone the previous evening showed a similar rise in the absorption of D as that found in II It therefore seems unlikely that these drugs inhibit the rise of D in the absorption test in alcoholics The reduced values found in I may be due to disturbances in the gastro-intestinal absorption of D or to processes involving the portal vein or liver Determination of D at 6 12 24 36 and 48 h showed that its elimination as reflected by its plasma half life was slightly slower in I than in II

225 Kanto J Iisalo E Lehtinen V Salminen J (Department of Pharmacology Turku University and Clinic of Psychiatry Turku University Central Hospital Turku Finland) CORRELATION BETWEEN PLASMA CONCENTRATIONS AND PSYCHIC EFFECTS OF DIAZEPAM IN LONGTERM TREATMENT

Plasma concentrations of diazepam (d) and N-demethyldiazepam (Nd) has been followed gaschromatographically for 5 24 weeks in 8 neurotic out patients receiving d 15 mg daily After the maximum concentration in 1-6 weeks (d 179 642 ng/ml and Nd 212 1313 ng/ml/ both d and Nd had a significant tendency to decrease (d 62 396 ng/ml and Nd 180-594 ng/ml) possibly due to a faster hepatic microsomal metabolism of d

The psychic condition was tested with Taylor s Manifest Anxiety Scale at intervals of one month and the condition was assessed both by the psychiatrist and a patient himself at intervals of one week The stepwise improvement in neurotic symptoms achieved by the therapy during the first month remained unaltered during the six following months After 4 6 weeks therapy no correlation was detected between d and Nd plasma concentration and psychic condition

The steady state concentrations of d and Nd in some other patients using d for 2 5 years were about 1/5-1/10 of those achieved by the same dose in an acute experiment In some of those patients the dose of d had partially lost its effectiveness after 0 5 2 years of therapy and the dose had to be increased

Hanhijärvi H Penttilä I Pekkarinen A & Hakulinen A (Department of Pharmacology University of Turku Finland) THE EFFECT OF ARTIFICIALLY FLUORIDATED DRINKING WATER ON THE PLASMA IONIZED FLUORIDE CONTENT IN CERTAIN DISEASES II

Plasma ionized fluoride concentration (IPF) was measured with the electrometric method in patients from areas with artificially fluoridated water (I) (1 ppm fluoride) and normal drinking water (II) A small but significant increase of IPF with advancing age was found in both areas IPF was higher in renal insufficiency (Acta Pharmacol Toxicol 31:104 Suppl I 1972) than in corresponding control

In cor pulmonale IPF values in area I 2 7 + 0 4 μM (6) (SEM no of patients) in the whole group of heart insufficiency 1 8 $\pm$ 0 1 μM (50) and in severe heart insufficiency 2 2 $\pm$ 0 3 μM (8) were significantly higher than in the corresponding control group of the same age 1 5 + 0 4 μM (178)

In a 6 years old boy with diabetes insipidus IPF in area I was 4 0 μM 4 times higher than in the control group of the same age In cirrhosis with ascites IPF was 2 0 $\pm$ 0 35 μM (3)

During pregnancy there was a significant decrease in IPF to 0 90 $\pm$ 0 04 μM (40) probably indicating an accumulation of fluoride in fetal or maternal bone When oedema complicated the pregnancy IPF was higher A slight but significant decrease to 1 2 + 0 051 μM (49) in hypertensive patients was probably due to treatment The conclusion is made that IPF is increased in diseases with marked generalized oedema This is in agreement with a previous study by us showing a marked elevation in renal insufficiency

Pekkarinen, A (Department of Pharmacology Turku University Finland) 227
THE EFFECT OF DIAZEPAM (D) ON THE HEART RATE (HR) DURING ORAL EXAMINATION OF PHARMACOLOGY (EP)

In 169 control students the mean HR at rest 70 4+0 6/min increased to 104 0+1 2/min (33 6+1 1/min) (p<0 001) during EP more in females (108 9+1 8/min N=61) than in males (100 9+1 5/min N 98)(p<0 001) The increase of HR was higher at the time of the first period of EP (11 8 min) and the first question (+37 7+1 1/min) than later (p<0 01) 5 mg D perorally at least 1/2 hr before the EP reduced only very slightly HR during EP to 96 9+1 6/min (p<0 001) and the rise of HR to +27 0+1 4/min resp (p<0 001) in 103 students as well as in both sexes in 35 females (to 102 0+2 6/min) (p<0 05) and in 68 males (to 94 3+1 9/min)(p<0 01) D (Valium) slightly reduced the rise of HR in the groups of moderate physical exercise (p<0 001) from 109+1 9/min (N=45) to 93 3+3 2/min (N 27)(p<0 001) or in physically inactive students from 107+2 3/min (N=46) to 101 5+2 3/min (N 33) with D (p<0 05) while D did not reduce further already low HR during EP 95 5+2 2/min (N=43) in the group of athletes students with active physical training or runners to 93 1+2 7/min (N 33) nor the rise of HR from +29 1+1 2/min to +27 8+2 3/min The rise of HR is higher during EP in the group of higher examination scores than in the group of lower examination scores (p<0 05) D reduced HR (p<0 01 and <0 001 resp) and the rise of HR (p<0 01 and <0 001 resp) in these two groups of students as well as in the students who had failed (p<0 05 p<0 05) or students who had passed their examination (p<0 01 p<0 01)

Parkki, M G and Irj la K (Depa tment of Phy iol gy University of Turku Finland): PURIFICATION OF GLYCINE N ACYLTRANSFERASE FROM RAT HEPATIC MITOCHONDRIAL FRACTION 228

Glycine-N cyltransf as (EC 2 3 1 13) atalys th glycine conjugation by condensing acyl coenzyme A with glycine to f rm the corresponding cylgly ine d ivative In rd r t tudy th substrat (salicyl t) induced activ tion f the onj g tion sy tem in detail (Irj 1 K Ann Acad Sci Fenn 1972 A V 154) fast a d efficient purifi ation p ocedure has been dev l ped

Rat liv mitochond i we e uspended in gly ine buffer (pH 9 6 ioni strength 0 1) co taining 1% rivanol They wer sonicated t liberate glycine-N acyltransf as from the parti les (Al o mitochond ial ceton powd r can be used as tarting mat rial and the nzyme can be extracted with the id f the ame buff) After edimenting the unsolubli ed prot in and rivanol-p t in complexes at 27000 g the supernatant containing glycine-N- cyltransfera e wa placed in a CM-Sephadex C 50 column equi libr ted with glycin buff r (pH 9 5 ioni strength 0 1) A convex NaCl gradient (from 0 to 0 2 mol/1) wa used in el tion The glycine-N-acyl transf ras was eluted a par t peak ft th main bulk of prot in The pecific tivity increased to 0 1 mol benzoyl gly ine formed/min g prot wich indicat s 200 fold purification compared with a crud mitochondrial extract Th enzyme pr par tion was very tabl (no hang in activity in four month at −20°C)

The pre ent method i simpl nd ep oducibl nd yields preparati in six hours pur enough for kineti tudi

229 Rane And rs (Depts of Pharmacol and Clin Pharmacol Karolinska Institutet Stockholm Sweden): N-OXIDE FORMATION FROM A TERTIARY AMINE IN HUMAN FETAL LIVER MICROSOMES

Human fetal liver microsomes contain an NADPH-dependent mixed-function oxidase system which catalyzes drug oxidations including C-oxygenations and N-dealkylations The present study was undertaken to see if human fetal liver microsomes could catalyze the N-oxygenation of a tertiary *amine N N-dimethylaniline* (DMA) *Human fetuses were obtained* via hysterotomy for the interruption of pregnancy for sociomedical reasons Gestational ages varied between 13 and 21 weeks The liver microsomes were found to N-oxidize DMA at rates varying between 0 3 and 2 4 nmoles DMA N-oxide formed/ mg protein x min^{-1} The reaction was dependent on NADP^{+} CO did not inhibit the reaction Rather an increased formation of DMA N-oxide (about 17 %) was observed when CO was present in the medium The findings are in agreement with results from N-oxygenation studies with rat and pig liver microsomes (cf Ziegler & Mitchell Arch Biochem Biophys 1972 150:116) These studies indicated that N-oxygenation of tertiary (and secondary) amines is independent of the cytochrome P-450 enzyme system

Various amines are known to exert toxic effects via their N-oxygenated metabolites Therefore N-oxygenation of amines in human fetal liver is of interest from a teratologic point of view

Supported by grants from Expressen s Prenatal Research Fund the Association f the Swedish Pharmaceutical Industry, and the Swedish *Medical Research Coun il (14 X- 3902)*

230 Harniemi, J and Hänninen, O (Department of Physiology University of Turku Finland): KINETIC PROPERTIES OF LIVER UDP GLUCURONYLTRANSFERASE WITH ^{14}C LABELED p-NITROPHENOL AS SUBSTRATE

The kinetic studies of UDP glucuronyltransferase have been hampered by the lack of a purified enzyme preparation free from contaminants and by the lack of a sensitive enzyme assay p-Nitrophenol has however been us d recently often as the aglycone substrate of the enzyme because of its easy and rapid pe trophotometric determination Unfortunately the method is not v ry a curate for the kinetic studies used

We have studied the kinetics of UDP glucuronyltransferase using ^{14}C labeled p nitrophenol as substrate *which increases the sensitivity* about 100 times compared with the spectrophotometric method The unconjugated aglycone and it glucuronide were separated from each other by the extraction with diethyl ther

Fresh and frozen and thawed mi rosomes of rat and guinea pig appeared to have a transition point in their Lineweaver-Burk plots when the UDP glucuronic a id concentration was varied leading to two Km-values for *UDPGlcUA Transition* as no observed with frozen microsomes of beef liver The reaction product uridin 5 diphosphate inhibited UDP glucuronyl transferase of frozen beef liver microsomes competitively with UDPGlcUA The results of this tudy ar in accordance with the findings of Winsnes (BBA 28 394 1972 BBA 289 88 1972) obtained with the spectrophotometric method

Hietanen E and O Hänninen (Department of Physiology University of Turku, Finland): EFFECT OF PROLONGED SALICYLIC ACID ADMINISTRATION ON THE MUCOSAL GLUCURONIDE SYNTHESIS IN THE RAT DUODENUM AND STOMACH 231

In previous experiments massive intragastric salicylic Acid administration three times at 12 hr intervals resulted in gastric hemorrhage together with decreased UDP glucuronyltransferase activity in the gastric mucosa (Aitio A. et al:Mucosal drug metabolism and drug-induced ulcer Int. Congress on Exp Ulcer Cologne 1972) The purpose of the present study was to follow changes in gastroduodenal glucuronide synthesis as related to the disappearance of gastric erosions in long-term salicylic acid treatment.

After a single dose of one mmole of salicylic acid per kg of body weight the mucosal erosions appeared in 12 hr and the UDP glucuronyltransferase activity decreased in the stomach. After three daily doses the erosions disappeared in most rats and the mucosal UDP glucuronyltransferase activity increased 2-fold in the duodenum and 1 5-fold in the stomach The increase was however transient, and the enzyme activity returned to the control level within two weeks The hepatic UDP glucuronyltransferase activity was also followed but the salicylic acid administration did not change it significantly The gastric and duodenal protein content decreased after a single salicylic acid administration but turned back to normal in three days despite of continuation of drug administration

The data show that during prolonged salicylic acid treatment the gastric erosions disappear and the mucosal glucuronidation capacity regenerates in the rat

Grant: U S Public Health Service (AM-06018-11)

Ravn Jonsen A (Department of Pharmacology University of Copenhagen Denmark): THE METABOLISM OF A N METHYLATED BARBITURATE (ENIBOMAL-^{14}C) IN THE RAT 232

In a recent investigation the distribution and excretion of unlabelled enibomal was reported (Ravn Jonsen Acta pharmacol, et toxicol 1973 32 401 407) Three metabolites were identified all derivatives of 5 isopropyl barbituric acid (B) 1 methyl 5 acetonyl B (II) 1 methyl 5 (2 -oxo 3 hydroxypropyl) B (VI) and 5 acetonyl B (VIII) Only 20 per cent could be recovered chemically in 72 hours

In the present investigation ^{14}C enibomal (ring labelled) was synthetized from ^{14}C urea. The labelled barbiturate was administered i v (45 mg/kg specific activity 0 2 mci/g) In a fractionated sampling period of 120 hours a total of more than 80% radioactivity was recovered with 70% in the urine 10% in faeces while only 0 3% was found in the expiratory air In the carcass was recovered 2% By TLC the same metabolites was demonstrated as mentioned above and further 1 methyl 5 carboxymethylene B (III) was identified Besides these ether extractable metabolites also the remnant contained unidentified radioactivity but treatment with urease did not liberate $^{14}CO_2$ Of the major metabolites VI dominated in the beginning VIII in the later part of the ampling period. All metabolites were demonstrated both in urine and faeces

233 Aitio A and Vainio H (Department of Physiology University of Turku Finland): INDUCTION OF MICROSOMAL XENOBIOTIC BIOTRANSFORMATION BY PHENOBARBITAL AND 3-METHYLCHOLANTHRENE ADMINISTERED BOTH SINGLY AND IN COMBINATION

The mechanism by which phenobarbital (PB) stimulates the microsomal drug metabolism has been considered to be different from the mechanism by which polycyclic carcinogens produce their induction In the present study we have examined the effect of phenobarbital (80mg/kg i p) and 3-methylcholanthrene (MC)(10 mg/kg in olive oil i p) when administered both singly and in combination on the hepatic microsomal drug oxidation (O-demethylation of p-nitroanisole hydroxylation of 3 4 benzpyrene) and glucuronidation (p-nitrophenol 4-methylumbelliferone) of the rat Both MC and PB increased the O-demethylation of p-nitroanisole (6-fold and 9-fold respectively) whereas only MC increased the benzpyrene hydroxyl se activity (13 fold) In combination their ffect was additive on p-nitroanisole demethylase activity where PB decreased the extent of MC induction of benzpyrene hydroxylase Both PB and MC enhanced the UDP glucuronyl transferase activity but no additive effect was obtained when th drugs were administered in a combination (calculated on microsomal protein basis) If calculated on total body basis an additiv effect was observed on UDP glucuronyltransferase activity

The two inducers used MC and PB thus seem to hav eith r additive inhibitory or no effect on each other s actions d pending on the enzyme studied

234 Arvela, P (Department of Pharmacology, University of Oulu, Finland): THE IN VITRO EFFECTS OF LANTHANONS ON LIPID PER- AND DRUG METABOLISM IN RAT

Lanthanons cause a severe liver injury which impairs the ug metabolizing capacity of rat liver (Arvela, P and Kärki, T , Experientia 1971 27 1189) The known hepatotoxicity of carbon tetrachloride (CCl_4) is supposed to be due to the enhanced lipoperoxidation in the liver (Recknagel, R O and Ghoshal, A K , Lab Invest 1966 15 132) In order to find out if lanthanons act like CCl_4 their effects in vitro on lipid peroxidation and drug metabolism were studied

The lipid peroxidation was measured as malonaldehyde liberated and for test-reactions of drug metabolizing activity the hydroxylation of 3,4-benzpyrene, the glucuronidation of p-nitrophenol the demethylation of aminopyrine and the amount of cytochrome P-450 were chosen The lanthanons studied were lanthanum, cerium and erbium

Contrary to CCl_4 all lanthanons studied inhibited the lipoperoxidation when incubated in vitro with liver preparations and none of the studied drug metabolizing enzymes were significantly inhibited by lanthanon concentrations up to 10^{-4} M

It is concluded that the toxic effects of the lanthanons are different in vitro than those of CCl_4 but more studies must be done to extend this assumption to in vivo circumstances

Grant: The Finnish Culture-Foundation, Finland

Jansen, J. Aas (Department of Pharmacology University of Copenhagen Juliane Mariesvej 2o DK 21oo Copenhagen Denmark): THE RATE OF DISSOCIATION OF SALAZOSULFAPYRIDINE FROM ALBUMIN BINDING 235

The rate of dissociation of drugs from plasma protein binding is generally assumed to be of such an order of magnitude that it does not limit the rate of renal tubular secretion However very little experimental data are available to prove or disprove this assumption.

The aim of the present investigation has been to determine the association (k_1) and dissociation (k_{-1}) rate constants of a drug-albumin binding Salazosulfapyridine (S) was chosen because its binding to human serum albumin (HSA) gives rise to a reduction in optical density at 32o nm The course of association of S to HSA was determined at 37° using a stopped-flow spectrophotometer (Durum) HSA from Behringwerke (2o μM) in a Tyrode solution (pH 7 4) was mixed with equal volumes of 2 μM - 4o μM S (donated by Pharmacia)

For three types of binding sites (Jansen, Acta Pharmacol Toxicol 1972 31 Supp I 88) k_1 and k_{-1} were determined by simulating the course of association on a digital computer using steps corresponding to 1 μsecond the three association constants and the number of sites of each type

At physiological and therapeutic conditions only binding to the sites with highest affinity are of importance The half-time of dissociation from these sites is calculated from k_{-1} to be o o8 - o 14 second As the passage time for the blood through the tubular capillaries is quoted to be about 2 5 second (Best & Taylor Baltimore 1961) the results indicate that the dissociation of S from HSA is not likely to limit the renal tubular secretion rate

Widman, M and Sandberg F (Department of Pharmacognosy Faculty of Pharmacy University of Uppsala Sweden): PLASMA PROTEIN BINDING OF 7 HYDROXY- Δ^1-TETRAHYDROCANNABINOL: AN ACTIVE CANNABIS METABOLITE 236

Earlier we have shown that Δ^1 tetrahydrocannabinol the major psychotomimetically active compound of Cannabis is extensively bound to the lipoproteins of human plasma 7 Hydroxy-Δ^1 tetrahydrocannabinol which is a pharmacologically and psychologically active metabolite of Δ^1 tetrahydrocannabinol in man, has been shown by equilibrium dialysis and ultrafiltration to be bound to 94-99% to plasma proteins Further experiments using the $[^{14}C]$ labelled compound, with agarose and polyacrylamide gel electrophoresis and ultrafiltration suggest that albumin α_1-lipoprotein and to a minor degree also β lipoprotein are involved in the protein binding of 7-hydroxy-Δ^1-tetrahydrocannabinol in blood plasma

237 Aitio A (Department of Physiology University of Turku Finland) DISTRIBUTION AND INDUCIBILITY OF UDP GLUCURONYLTRANSFERASE IN DIFFERENT RAT TISSUES

Liver has been regarded as the main site of glucuronide formation though UDP glucuronyltransferase activity has been d tected also in the kidney skin and gastrointestinal mucous membrane Recently also lung has been demonstrated to possess transferase activity (cf Aitio Xenobiotica 1973 in pr ss) Therefore a reinvestigation of the presence and ind cibility of UDP gl curonyltransferase in different rat tissues was undertaken

A low UDP glucuronyltransferase (4-methylumbelliferone) activity was detected in the brain retroperitoneal fat and cardiac and diaphragmatic muscle and adrenal gland An intermediate activity was foubd in the thymus spleen and lung A high activity resided in the g strointestinal mucosa kidney and liver The blood was totally devoid of activity A low activity wa demonstrated in the placenta too Drug induced rise of UDP glucuronyl transferase activity was studied in the kidney liver lung sple n and thymus Treatment with cinchophen resulted in an increase of the activity in the lung liver and kidney whereas 3-methylcholanthrene caused an increase in the liver and lung only Th inducing power of phenobarbital was restricted to the liver and chlorpromazine was devoid of inducing potency

Thus all the portals of foreign compounds to the body as well most other org ns possess at l ast some capa ity to transform xenobiotics to glucuronides which are water soluble and thus easily excreted

Grant: U S Public Health S rvice (AM-06018 11)

Klausen M (Laboratory of Pharmacology Royal Danish School f Pharmacy Copenhagen Denmark): RAPID TRANSIENT TOLERANCE MICE TO PENTOBARBITAL

?e mice given 3o mg/kg pentobarbital i p show a plasma concentration of 15-2o µg/ml after 3o min They are anaesthetized and unable to run on the rotacone (J D Christensen Acta Pharmacol in press) Re-administration of the same dose after 12-19 hours gives a decreased anaesthesia and in some mice a nearly normal motor coordination measured on the rota cone after 3o min The repeated dose 24 hours after the first dose shows a le s pronounced tolerance than that observed after 12-19 hours The pentobarbital plasma concentration f the mice made tolerant is correspondingly diminished so that the observed tolerance is mainly metabolic

AUTHOR INDEX

The figures refer to the abstract numbers

Aakvaag, A. 100
Aaren, G 122
Aarseth, P 78, 82, 151
Aarseth, S. 151
Ahlenius, S 115, 123
Ahren, K. 18, 158
Aitio, A. 233,237
Anstorp, O. 74
Andén, N. E. 110, 115
Andersen P 38
Anderson, P 201
Andersson, R. 3
Andersson, S.A. 175
Antelman, S. 112
Appelberg, B. 92
Arvela, P 234
Aquilonius, S. M. 116
Atkins, J.L. 141
Aunaes, I. 80
Avenno, A. 112

Batra, S. 213
Bergan, A. 151
Berglind, T 83
Bergstrøm, S. 14
Bie, P 31
Binkhorst, R.A. 98
Bjørklund, A. 33b, 59
Blix, A.S. 144
Bojesen, E. 32
Bolme, P 152
Bonde-Petersen, F 98
Borg K.-O 219
Borgå, O 27, 28
Bradshaw S.D 211
Brightman, M. W 9
Brown, R. 125
Burg, J van der 95
Buus Larsen, J 124 134
Bydgeman, M. 16
Bø, G 82, 157

Carlson, L.A. 192
Carlsson, A. 47, 72, 114 125
Carlsson, E. 153
Carrer H. 128
Chakravarty N. 200
Christensen, A.V 113
Christensen, S. 210
Christoffersen, T 2 5
Corrodi, H. 111

Dahl, S.G 29
Dahlöf, C. 153
Delius, W 143

Diamant, B. 198
Duttmann, L. 174
Dyblog E. 218
Dyhre-Poulsen, P 93
Døving, K.B. 58

Eckernäs, S.-Å. 116
Edman, K.A.P 190, 196
Edstrøm, A. 171
Edwall, L. 35
Eide, I 138
Ek, L. 133
Ekelund, M. 91
Eklund, B. 146
Eliasson, M. 129
Eliasson, R. 16, 172
Eneroth, P 16
Engel, J 115 123 125
Ericson, L.E. 11
Ericsson, E. 6
Eriksson, E. 11
Essén B. 104
Essen, D O.Van 53

Fadnes, H.O 79
Fagrell B. 155
Felding, P 198
Fjalland, B. 188
Folkow B. 41
Forsyth, R.P 152
Frederiksen, O 163
Fremstad, D 26
Frenning, B. 86
Frey, H.-H. 167
Fri, C.-G 71
Fuxe, K. 60 111
Fyro, B. 73
Fänge, R. 166

Galbo, H. 195
Garberg, L. 219
Gautvik, E.L. 144
Goldstein, M. 60, 111
Gollnick, P O 103, 108
Groth, T 13
Grove, D 134
Gustafsson, L. 169

Haegerstam, G 53
Haffner J.F W 214
Haglund, U 104
Hak, P 95
Hakulinen, A. 226
Hamberg, M. 22
Hanhijärvi, H. 226

Hansen, A.J 187
Harrl, M.N.E. 191
Hauge, A. 157
Hedquist, P 17 22, 169 173
Helgason, S. St. 182
Henriksen, O 145 156
Herlitz, H. 189
Hermansen K. 131
Hertz, L. 174
Hietanen, E. 231
Hirwonen, L. 109, 149
Hlastala, M.P 106
Holler B. 177
Holmgren, S. 160
Hope, D.B. 184
Hounsgaard, J 93
Hultborn, R. 189
Hyvärinen, J 40 148
Hänninen, O 230
Hakfelt T 60, 61 111

Iitalo, E. 225
Ilebekk, A. 193
Irjala, K. 228
Irvine, D.R.F 51
Isaksson, O 194
Ishizaki, T 152

Jacobsen, S. 24
Jansen, J.Aas 235
anson, P O 18, 158
enekog T 96
ohannessen, J 204
ohansen, K. 39
ohansen S.H. 101
Johansen, T 200
ohansson, B. 10
Johansson, H. 94
ohanmson, M. 190
ohansson, R. 165
ohnsen, Øyvind 172
onsson O 63, 178
Junstad, M. 23
ergensen, K.E. 140
ärvensivu, P 149

Kaljser L. 146, 192
Kaltiala, E. 222
Kanje, M. 171
Kanto, J 225
Karlmark, B. 207
Karlsson, J 102
Karvonen, J 107
Kehr W 72, 114
Kelly P J 53
Kerttu, I. 228
Kill, F 33 137 138, 204
Kinemuchi, H. 186, 188
Klausen, M 238
Klootwijk, R. 95
Knapp T.M 119
Knibestøl, M. 56

Koefoed Johnsen, V 161
Krarup, N 150, 159
Kärki, N T 222

Lagerkrantz, H. 169 216
Laitinen L. 191
Lammert, O 99
Landmark, K. 144
Lang L. 162
Larmi T K.I. 222
Larsen, J.A. 150 159
Larsen, J J 131
Larsen, N Ruberg 98
Larsson, G. 15, 19
Lassen, N.A. 13, 77
Lassers, B.W 192
Lehtinen, V 225
Leissner P.E. 175
Lekven, J 193 197
Leppäluoto J 181
Lenlant, G.J 106
Lidbrink, P 111 121
Lie, M. 204
Lindbom, L.-O 139
Lindholm A. 105
Lindholmer C. 172
Lindquist, M 72, 114
Lindvall O 59
Lisander, B. 11, 153
Livesey, P J 120
Ljung, B. 147
Ljungberg, T 117
Ljungdahl, Å. 61
Lorens, S.A. 118
Lowy J 46
Lubar J F 119
Lukkari J 97
Lund-Andersen, H. 75
Lundegård, K. 91
Lundgren, O 49 194
Lundholm, L. 1
Lybeck, H. 181
Lyon, I 161
Løfstedt, L. 143
Løw H. 192
Løyning E. 158

Magnusson, T 110
Malmnäs, C.O 133
Mandel, P 174
Marniemi, J 230
Martner J 153
Mattiazzi, A. 196
Melander A. 203
Mellander, S 50
Melmon, K. 152
Meyer P 120
Meyerson, B.J 122 128, 129 153
Miller S. 93
Mjørndal, T 223
Molbech, S. 101
Moore, R.Y 35b

Mustaparta, H. 57
Myhre, H.O. 144
Myrhage, R. 11
Mäkitalinen, M. 109
Møller J V 140
Møller M. 7, 75, 185
Møller, O J 205
Møllgård, K. 7 8, 185

Nesheim, B. I 214 125
Nicolaysen, G 12
Nielsen, G Damgård 88
Nielsen R. 164
Nielsen, S.E. 77
Nielsen, S. Levin 81 156
Nilsen, O.G 25
Nilsson, E. 196
Nilsson, G 83, 84
Nilsson, K. 4
Nilsson, S. 154 160
Nissen, J 198
Nordberg, A. 69
Nordeng, H. 58
Norn, S. 198

Oakley, B. 58
Odar-Cederlof, I. 27
Olbe, L. 219
Olgart, L. 55
Olsen, I. 89
Olsen, N 198
Olson, L. 62, 64
Olsson, K. 30
Omvik, P jr 137
Oreland, L. 186, 188, 223
Osnes, J.B. 2, 5
Otto, U 150

Paalzow G 70
Paalzow, L. 70
Pasako, W.P 81 145 156
Palmer L. 220
Parkki, M. 228
Parving, H.-H. 77
Paulev, P.-E. 195
Pekkarinen, A. 224 226, 227
Pelkonen, O 222
Peltonen, T 109
Pentilä, I. 226
Pernow B. 104
Persson, A.E.G 135, 207
Persson, N-A. 173
Persson, S.-A. 126
Petersson, G.O G 199
Piehl, K. 103
Piene, H. 82
Pinter G G. 141
Poranen, A. 54
Poulsen, H.L. 78
Pycock, C. 178
Pyykkö, I. 148

Raljola, E. 224

Rane A. 229
Ranje, C. 127
Rasmussen, S 87, 88
Ravn-Jonsen A. 232
Refsum, H. 144
Rosell, S. 48
Rossing, N 77
Russell J T 183
Rustwurm, H 58
Ræder M. 137
Røed, A. 180

Sachs, C. 63 178
Salminen, J 225
Saltin B. 104
Sandberg F 236
Schnermann, J 135
Schubert, J 73
Schäfer A. 167
Secher, N 98
Sedvall, G 71, 73
Seiger A. 62, 64
Sejrsen P 156
Sellman, R. 224
Selstam, G 158
Sensenbremer M 174
Serck-Hanssen, G 217
Setekleiv J 214
Sheikh, M.I 140, 209
Siesjø, B.K. 43
Siggins, G 177
Sillvenius, H. 94
Sivertsson, R. 147
Sjøberg, E. 162
Sjødin, L. 84
Sjøstrand, S.E. 219
Sjøstrøm, M. 165
Skadhauge, E. 211
Skaugen, E. 179
Skinner S.L. 208
Skoglund, C.R. 162
Skrede, K.K. 176
Slorach, S.A. 201
Solteil, M. 206
Sovijärvi, A.R.A. 52
Squires, R.F 66
Stenevi, U 35b
Stjärne, L. 170
Stock, G 110
Storm-Mathiesen, J 37
Strandberg, K. 21
Stromme, S.B. 100
Sundell, G 219
Sundler F 203
Sundlaf, G. 143
Sundwall, A. 69 116
Svanborg, N. 22
Svedmyr, N. 221
Søgnen, E. 89
Søndergaard, J 20
Sørenby L. 202
Sørensen, S.C. 7 8, 74, 185, 187

Teyler, T 38, 176
Thesleff, S. 44
Thiringer G 221
Thorn, N.A. 183
Thornell L. E. 165
Timisjärvi, J 149
Tindall A. 90 142
Tirri, R. 191
Tuomisto, J 65
Tuomisto, L. 67

Ulfendal H.R. 139 141 206
Ungerstedt, U 111, 112, 117 127 177
Ussing, H.H 45 161
Uvnäs, B. 201

Vaage, J 80
Vainio, H. 233
Vale, H. 118
Vallbo, Å.B. 42
Vilhardt, H. 184
Virkkunen P 181

Waaler B.A. 76, 157
Wahlqvist, M. 192
Wahlstrom, G 126, 132
Waller M. 85
Wallin, G 143

Walum, E. 171
Welss, O.B. 212
Wennmalm, Å. 23
Wester K.G 38, 51
Westergaard, E. 9
Westgren, U 203
Widman, M. 236
Wiesel, F.A. 71
Winbladh, B. 68
Wistrand, P 207
Wolgast M. 141
Wranne B. 106

Yoo, Y.B. 186

Ziebell A. 198
Zimmer J 56

Änggård, E. 15, 19

Oberg, B. 34
Obrink, K.J 85, 91
Øye, I. 2 5

Åblad B. 153
Agerup B. 135, 136, 141 207
Akerlund, G 166

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM
397

ON THE TURNOVER OF ACETYLCHOLINE IN THE BRAIN

AN EXPERIMENTAL STUDY USING INTRAVENOUSLY INJECTED RADIOACTIVE CHOLINE

By
Bengt Sparf

STOCKHOLM 1973

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM
397
From AB KABI Research Department, Pharmacology and Toxicology
S-104 25 Stockholm 30, and Department of Pharmaceutical
Pharmacology University of Uppsala, S-751 23 Uppsala
Sweden

ON THE TURNOVER OF ACETYLCHOLINE IN THE BRAIN

AN EXPERIMENTAL STUDY USING INTRAVENOUSLY INJECTED RADIOACTIVE CHOLINE

By
Bengt Sparf

Printed in Sweden 1973
KåWe Composer & Fotosats, Uppsala

The present survey is based on the following papers and some unpublished results

I B Sparf and J Schuberth Metabolic disposition of radioactive choline in the mouse with a special reference to the transport from plasma to brain. Submitted for publication in Eur J Pharmacol.

II. L. Ewetz, B. Sparf and B. Sörbo Enzymatic determination of choline in brain with choline phosphokinase and ^{32}P-labelled ATP Symposium on *In vitro* Procedures with Radioisotopes in Medicine In Vienna, 1969 Ed. *International Atomic Energy Agency (1970). SM–124/39 175–183*

III J Schuberth, B Sparf and A. Sundwall A technique for the study of acetylcholine turnover in mouse brain *in vivo*. J Neurochem. *16* (1969) 695–700.

IV J Schuberth, B. Sparf and A. Sundwall On the turnover of acetylcholine in nerve endings of mouse brain *in vivo*. J Neurochem. *17* (1970) 461–468.

V S M. Aquilonius, F Flentge, J Schuberth, B Sparf and A. Sundwall. Synthesis of acetylcholine in different compartments of brain nerve terminals *in vivo* as studied by the incorporation of choline from plasma and the effect of pentobarbital on this process. J Neurochem. *20* (1973) 1509–1521

VI. B. Lundholm and B. Sparf The effect of atropine on the turnover of acetylcholine in the brain. Submitted for publication in Eur J Pharmacol.

In the following, the investigations are cited by their Roman numerals.

CONTENTS

	page
1 INTRODUCTION	7
2 GENERAL APPROACH	10
2.1 Turnover principles	10
2.2 Determination of choline in the brain	11
2.3 Determination of acetylcholine	15
2.4 Separation of radioactive choline metabolites	15
3 TRANSPORT OF CHOLINE FROM PLASMA TO BRAIN	17
3.1 The fate of choline in plasma	17
3.2. The source of brain choline	18
4 TURNOVER OF ACETYLCHOLINE IN THE BRAIN	23
4.1 Estimation of turnover rate of acetylcholine	23
4.2. Compartmentation of newly synthetized acetylcholine	26
5 EFFECT OF DRUGS ON THE TURNOVER OF ACETYLCHOLINE	31
6 SUMMARY	40
7 ACKNOWLEDGEMENTS	42
8 REFERENCES	43

I INTRODUCTION

Biosynthesis of acetylcholine (ACh) in brain tissue was demonstrated as early as 1936 by Quastel and co-workers by using slices from brain cortex. In 1943 Nachmansohn and Machado showed that extracts from brain tissue could synthetize ACh in the presence of choline (Ch) under anaerobic conditions without glucose, if adenosine-5'-triphosphate (ATP) was added. The enzyme system which could synthetize ACh from Ch was named cholinacetylase. The significance of acetylcoenzyme A (acetyl-CoA) for the synthesis of ACh from Ch was described by Korkes *et al.* 1952. When subsequently it was generally accepted that the acetylation of Ch with acetyl-CoA occurred in two steps, the enzyme catalyzing the last step (see below) was called choline acetyltransferase (ChAc) (EC 2.3.1.6). This term had previously been introduced by Nachmansohn (1951).



$$HOCH_2CH_2\overset{+}{N}(CH_3)_3 + CH_3COSCoA \xrightarrow{ChAc} CH_3OCOCH_2CH_2\overset{+}{N}(CH_3)_3 + HSCoA$$

In 1956 Hebb showed that 60 % of the ChAc activity of a brain homogenate was localized in the mitochondrial fraction after centrifugation and the rest in the supernatant. An important subsequent discovery was that the nerve terminals in the brain withstand homogenization in sucrose. They became detached but seem to remain intact in isotonic media. These nerve ending particles or synaptosomes have been isolated by differential and gradient centrifugation (Whittaker 1959). Several investigations have shown that ACh (~ 80 %) and ChAc (~ 60 %) are localized in the nerve ending fraction (Hebb and Whittaker 1958, Hebb and Smallman 1956).

Further methods were developed by de Robertis *et al.* (1963) and Whittaker, Michaelson and Kirkland (1964) to isolate the vesicles, which have been observed in the nerve ending particles by electron microscopy. These vesicles, some of which contain ACh, could be released from the nerve endings by hypoosmotic shock. De Robertis postulated that the vesicles were both the site of synthesis and the storage place of the transmitter.

Whittaker believed that the vesicles only were the storage place of the ACh. These different opinions were based on the supposition that ChAc is localized in the vesicles (de Robertis *et al.* 1963, McCaman, de Lores Arnaiz and de Robertis 1965) or that it is soluble in the cytoplasm of the nerve ending (Whittaker 1964). Further, it has been suggested that the enzyme is bound to a non-vesicular

component (Tuček 1966). Later it was shown that pH and ionic strength are of great importance for the binding properties of ChAc to the vesicles and other membrane fragments (Fonnum 1967). By extrapolating to *in vivo* conditions it was assumed that the enzyme is normally in free form in the cytoplasm

A relatively large number of drugs with various pharmacological action have been shown to influence both the release of ACh from different cortical areas and the amount of ACh in the brain (Mitchel 1960 Bartolini and Pepeu 1967 Richter and Crossland 1949 Holmstedt, Lundgren and Sundwall 1963 Giarman and Pepeu 1962 Pepeu 1963 Consolo *et al.* 1972). However no attempts have been made yet to study the effects of drugs on the turnover of ACh in the brain. This would provide valuable information on the regulation of the synthesis, storage and release of ACh. The aim of the present investigation has been to study the turn over of ACh in the brain, particularly how pharmacological agents interfere in this process.

As the brain seems to be incapable of synthetizing Ch *de novo*, because the brain tissue cannot convert ethanolamine lipids into Ch lipids by stepwise methylation of the base (Bremer and Greenberg 1961 Marshall, Chojnacki and Ansell 1965 Ansell and Spanner 1967 1968), Ch has to be supplied to the brain from plasma. Consequently it seemed expedient to use intravenously administered labelled Ch as the precursor It was therefore necessary to know the fate of the precursor in the plasma and to separate and identify the radioactive Ch metabolites (cf Fig. 1) (I). To enable measurement of the specific radioactivity (SA) not only of ACh but also of Ch, much work has been done to develop methods for the determination of endogenous Ch in the brain (II III). This made it possible to estimate the turnover of ACh in the whole brain and in the nerve endings by using intravenously injected labelled Ch (III, IV). The localization of labelled Ch and ACh in subcellular fractions of brain nerve endings was studied in paper V The functional importance of the findings has been investigated by study ing the effects of drugs, such as pentobarbital, oxotremorine and atropine, on the parameters (III VI and unpublished results).

METABOLISM OF CHOLINE

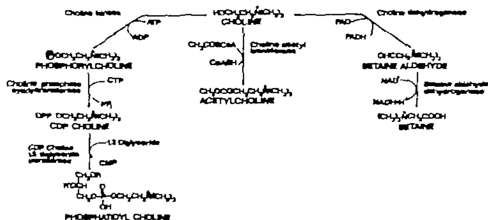


Fig. 1 Metabolic pathways of choline.

2. GENERAL APPROACH

2.1 Turnover principles

There has been considerable confusion concerning the turnover terminology. However, Zilversmit has proposed the following definitions. *Turnover rate*: The quantity of material turned over per unit of time. The meaning of turnover rate is unequivocal only when a steady state exists, i.e., when the rate of synthesis and transport into a compartment equals the rate of breakdown and exit. *Turnover time*: The time required for the turnover of an amount of material equal to the pool size (Zilversmit 1955).

When studying the turnover of a substance much information is obtained by establishing a precursor product relationship. The following three criteria for an immediate precursor are most used (Zilversmit 1943).

i) The specific radioactivity (SA) of the immediate precursor is greater than that of its product before the latter reaches its maximum SA.

ii) After the product has reached its maximum SA, the slope of the SA time curve of the product is negative and therefore the SA of the compound is greater than that of its precursor.

iii) At the time when the product has reached its maximum SA the slope of the SA-time curve of the product is zero and therefore the SA of the immediate precursor equals the SA of the product at that time.

In the present study the ACh turnover in the brain has been estimated by measuring the isotopic dilution of the precursor Ch in the brain and the initial rate of the radioactive ACh formation after intravenous injection of labelled Ch. This estimation of the ACh turnover is based on some assumptions which are summarized below.

i) The brain Ch is localized in an open single compartment system.

ii) There is a rapid equilibration of the injected radioactive Ch with the endogenous store of Ch.

iii) The synthesis of ACh from Ch is the rate limiting step (not the transport of Ch to the site for ACh synthesis).

All these assumptions are probably not fulfilled but the approach may nevertheless be valuable as the main intention is to use this technique to detect

changes in turnover rate of ACh induced by drugs. The turnover has also been calculated from the decay curve of the labelled ACh. However the results are influenced by concomitant synthesis of ACh. As will be described in chapter 4 there is probably also a re-uptake and re-use of radioactive Ch from released and hydrolyzed ACh. These difficulties might at least partly be overcome by blockade of ChAc but at present there is no ChAc inhibitor which functions satisfactorily *in vivo*. Therefore, this approach will result in an underestimate of ACh turnover

Another way to investigate the effect of a drug on the ACh turnover is to inject the drug intravenously after the injection of the radioactive Ch when (^{3}H)-ACh has reached its maximal level in the brain. The effect on the ACh turnover could then be estimated by comparing the decay curves of (^{3}H)-ACh in the brains of the control and the drug treated animals (VI).

2.2 Determination of choline in the brain

The difficulties in assaying free Ch in the brain can be seen from Table I, where values of the Ch content of the brain, which have been reported in the literature are tabulated. Besides the differences in the specificities of the methods, there are other difficulties in estimating free Ch in the brain. Thus, less than 1 % of total brain Ch (~ 20,000 nmoles/g) is free Ch (cf Schuberth, Sparf and Sundwall 1970 a). Further glycerophosphorylcholine (GPhCh) is present in the brain in about 400 nmoles/g (Ansell and Spanner 1970). As shown in Fig. 2, GPhCh can also be formed from both Ch plasmalogen and lecithin in animal tissue (Dawson 1966). GPhCh can split off Ch in brain tissue, a reaction catalyzed by GPhCh diesterase (Webster Marples and Thompson 1957), and it is readily hydrolyzed to yield Ch under acidic conditions (Appleton *et al.* 1953). Thus, there are possible errors due both to killing techniques (enzymatic hydrolysis before denaturation of diesterases) and to extraction procedure (acid hydrolysis due to e.g., the acid protein precipitating agent) which must be considered. A rapid postmortal increase of endogenous free Ch does in fact occur in brain tissue if the brains are not frozen rapidly enough (II IV and Dross and Kewitz 1972).

As the methods previously used for Ch estimation in tissues (e.g., before 1967 when this work started) are rather unspecific and circumstantial a new method was developed (paper III). The method, which was the first enzymatic assay for Ch is based on the ChAc catalyzed formation of labelled ACh from labelled acetyl-CoA and the Ch in the extract.



$$(CH_3)_3NCH_2CH_2OH + C^3H_3COSCoA \rightarrow (CH_3)_3NCH_2CH_2OCOC^3H_3 + CoASH$$

Table I
Summary of determinations of free Ch in the brain

Authors	Publishing Date	Material	Method	Content nmoles/g	(n)
Smith & Saelens	1967	Rat brain	ChAc	600	
Ansell & Spanner	1968	Rat brain	Periodide	170 ± 10	(5)
Hebb	1968	Rat brain	Biological	200	
Marchbanks	1968	Guinea pig cortex	Biological	220	
Schubert, Sparf & Sundwall	1969	Mouse brain	ChAc	115 ± 2.4	(5)
Ewetz, Sparf & Sörbo	1969	Rat brain	Ch kinase	39.3 ± 0.85	(10)
Ewetz, Sparf & Sörbo	1969	Mouse brain	Ch kinase	43.0 (41.4 45.5)	
Saelens, Allen & Simke	1970	Mouse brain	ChAc	131.5 ± 5.3	(27)
Hanin, Masarelli & Costa	1970	Rat brain	Gas chromatography	274 ± 16	(11)
Schubert & Sundwall	1971	Mouse brain	ChAc	68.1 ± 6.11	(10)
Reid, Haubrich & Krishna	1971	Rat brain	Ch kinase	67.8 ± 4.8	(7)
Collier Poon & Salehmoghaddam	1972	Mouse brain	Biological	68.8 ± 6.4	(9)
Consolo, Ladinsky Peri & Garattini	1972	Mouse brain	ChAc	62.7 ± 2.0	(4)
Browning	1972	Rat brain	Fluorometry	107 ± 8	(4)
Dross & Kewitz	1972	Rat brain	Biological	42	
Dross & Kewitz	1972	Rat brain	Biological	27.5*± 1.7	
Eade, Hebb & Mann	1973	Rat brain	Biological	21.5 ± 5.1	(10)
Eade, Hebb & Mann	1973	Rat brain	ChAc	29.1 ± 10.1	(11)
Eade, Hebb & Mann	1973	Rat brain	Ch kinase	35.0 ± 11.0	(4)

*) Extrapolation to *in vivo* conditions from postmortal increase of free Ch.

The labelled ACh formed was determined by radioassay after separation from the excess of labelled acetyl-CoA. While the present study was in progress the same principle was published by Smith and Saelens 1967 who found the brain to contain about 600 nmoles Ch/g of brain tissue. By our version of the ChAc method a Ch value of 115 nmoles/g was obtained (III).

At about the same time two other reports on the Ch content of the brain were published. By using a periodide method (Ansell and Spanner 1968) and a biological method (Hebb 1968), the brains were found to contain 170 nmoles/g and 200 nmoles/g respectively. Owing to the different values obtained a new principle was used (II). It is based on the Ch phosphokinase reaction and radioassay of the radioactive PhCh formed from the reaction between added AT^{32}P and Ch in the extract.

CH_2OR / $R'OCH$ / $CH_2OPOCH_2CH_2N(CH_3)_3$ (O, OH) — Lecithin

$CH_2OCH{=}CHR$ / $R'OCH$ / $CH_2OPOCH_2CH_2N(CH_3)_3$ (O, OH) — Chol plasmalogen

Sphingomyelin

Ceramide

Fatty acid

CH_2OR / $HOCH$ / $CH_2OPOCH_2CH_2N(CH_3)_3$ (O, OH) — Lysolecithin

$CH_2OCH{=}CHR$ / $HOCH$ / $CH_2OPOCH_2CH_2N(CH_3)_3$ (O, OH) — Lysocholine plasmalogen

(P)$OCH_2CH_2N(CH_3)_3$ — Phosphorylcholine

Fatty acid

RCH_2CHO Fatty aldehyde

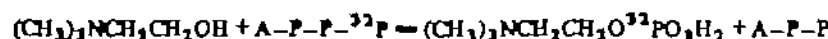
CH_2OH / $HOCH$ / $CH_2OPOCH_2CH_2N(CH_3)_3$ (O, OH) — Glycerylphosphoryl choline

CH_2OR / $R'OCH$ / CH_2OH — 1,2 Diglyceride

Choline

CH_2OH / $HOCH$ / CH_2O(P) — 3-Glycero phosphate

Fig. 2. Breakdown of choline containing phospholipids.



$$(CH_3)_3NCH_2CH_2OH + A{-}P{-}P{-}^{32}P \rightarrow (CH_3)_3NCH_2CH_2O^{32}PO_3H_2 + A{-}P{-}P$$

By this method rat brain was found to contain 39.3±0.95 nmoles Ch/g and mouse brain 43 0 nmoles/g. As the above Ch values were lower than had been reported previously with other methods (Table I) a number of control experiments were performed (II).

There are, however at present also some practical disadvantages in its rather

low sensitivity and because the Ch phosphokinase is rather unstable. However smaller amounts may be estimated with a higher SA of $AT^{32}P$ (0 05 mCi/mM) or by decreasing the incubation volume (2 ml). An advantage is that the enzymatic phosphorylation reaction can be carried out under extremely mild conditions which, as mentioned, is important for the estimation of Ch in the brain.

Recently two further methods, in which Ch kinase is employed for the determination of both ACh and Ch have been published. One involves a preliminary separation of ACh and Ch by electrophoresis (Reid, Haubrich and Krishna 1971). By this method about 0 4 nmoles/g brain could be measured and the rat brain was found to contain 65 nmoles Ch/g. The other method uses reactions which are catalyzed by the following enzymes ACh-esterase, Ch phosphokinase pyruvate kinase and lactate dehydrogenase (Browning 1972). By this procedure 0 1 nmole of Ch and ACh could be detected and the Ch content of the brain was estimated at 106 nmoles/g. The advantage of these new assays using Ch phosphokinase, is their higher sensitivity in comparison with the original Ch phosphokinase method at present. However they also give somewhat higher values than the method described in paper (II) (cf Table I) which seems to be one of the most thoroughly checked (II).

By modifying our original ChAc method, the Ch values decreased from 115 nmoles to 68 nmoles Ch/g. The same principle used by Consolo *et al* (1972) gives 62.7 nmoles/g

From the discussion above it seems obvious that there is no single simple explanation for the varying Ch values reported for brain tissue. Very recently Eade Hebb and Mann (1973) have reported a comparison of three different methods based on different principles. A biological method (determination of ACh on the rectus abdominis of the frog after acetylation of Ch in the extract with acetylchloride) was run parallelly with a modification of the Ch phosphokinase method (II) and a ChAc assay The Ch phosphokinase method confirmed the low value obtained in paper II but the ChAc assay and the bioassay gave still lower values (29 and 22 nmoles/g respectively).

By combining a precipitation reaction (e.g., reineckate) and gas chromatography or gas chromatography/mass spectrometry methods have been developed with a high sensitivity for determination of the SA of Ch and ACh in turnover studies with (^{1} C) and deuterium Ch as ACh precursors (Hanin, Massarelli and Costa 1972 Jenden 1972) These new methods have probably great potential possibilities in the future but the costs may be a limitation. Methodological difficulties have been present resulting in too high Ch values of the brain (cf Table I).

To sum up, it may be concluded that many of the values published (Table I) are too high owing to factors such as the postmortal increase in free Ch, hydrolysis of Ch containing compounds by the acid extractants or too low specificity of the methods. The exact value of free Ch in the brain is, therefore, not known, but all evidence taken together indicates a Ch value in the range of 40±20 nmoles/g. This value (40 nmoles/g) has been used in this work for calculations of the SA of Ch in brain.

2.3 Determination of acetylcholine

The brains were homogenized in trichloroacetic acid which was removed by extraction with ether. The endogenous ACh in the extract was measured on a small strip of the dorsal muscle of the leech (Szerb 1961). When known amounts of ACh were added to the tissue fractions no potentiation or inhibition was observed. Further insignificant contractions were obtained by samples treated with alkali (pH 11–12) for 30 min at room temperature. In the presence of d tubocurarine (10^{-5}M) less than 10 % of the original ACh-like activity of the extracts were obtained. Ch was about 1,000 times less active than ACh on the leech muscle. By the present technique 0.5–5 pmoles of ACh in a tissue extract can be determined. The mouse brain was found to contain 16.9±1.52 nmoles/g (mean±S.E. n=6). This value is in agreement with those of Karlén *et al.* (1973), who compared biological estimation of ACh with methods based on gas chromatography/mass spectrometry and pyrolysis gas chromatography (Karlén *et al.* 1972).

2.4. Separation of radioactive choline metabolites

For the present study an efficient separation of the Ch metabolites (Fig. 1) was essential. This was initially obtained by isolating the quaternary ammonium compounds in the brain tissue extracts by reineckate precipitation followed by exchange of the reinecke ion for chloride on a Dowex–X8 anion column. Labelled Ch and ACh was then separated by high voltage electrophoresis at pH 4.6 in 0.2 M acetate buffer (Heilbronn and Carlsson 1960). A disadvantage of this system, used in papers III and IV and partly in paper I, is that the desalting step with reineckate precipitation is rather time consuming and that PhCh is lost as it is not precipitated with reineckate.

For these reasons the high voltage electrophoresis system was modified by a buffer of the following composition pyridine:acetic acid:acetone:water

(8.8.30 154 pH 4 6) (I). Using this system Ch, ACh, PhCh, GPhCh and cytidyldiphosphorylcholine (CDPCh) can be separated in concentrated trichloroacetic acid extracts. By complementing this system with high voltage electrophoresis at pH 10 and acetylcholinesterase (AChE) hydrolysis of the ACh spot obtained at electrophoresis at pH 4 6 the following substances could be separated Ch, ACh PhCh, betaine aldehyde, betaine, GPhCh, CDPCh and lecithin. Reproducible recoveries in the range of 85–90 % were achieved for Ch, ACh, PhCh and betaine. The tailing from the Ch to the ACh spot was less than 0.5 % on electrophoresis at pH 4 6. Since complex binding of TCA to quaternary ammonium compounds has been reported to influence the migration of Ch-metabolites in chromatography systems (McCarthy Knight and Chenoweth 1973), the recoveries of the different Ch metabolities were carefully checked during different conditions (I and III).

3 TRANSPORT OF CHOLINE FROM PLASMA TO BRAIN

3.1 The fate of choline in plasma

Free Ch in the brain is probably used as the precursor of both ACh and phospholipids. However as previously mentioned, the brain appears unable to synthetize Ch *de novo* and should therefore be dependent upon an exogenous supply of Ch.

The only known pathway for *de novo* synthesis of Ch in higher animals is the stepwise methylation of phosphatidylethanolamines by adenosylmethionine to form phosphatidylcholine (lecithin) (Bremer and Greenberg 1960). This route was shown to be of significance for the biosynthesis of lecithins in the liver (Bjørnstad and Bremer 1966). Estimates by Wise and Elwyn (1965) indicate that this pathway may provide Ch in amounts equivalent to the dietary intake of the rat, or about 13 μmoles per day and gram liver. Lecithins may also be formed by incorporation of preformed free Ch via PhCh and CDPCh (Fig. 1 Kennedy and Weiss 1956). That the latter route is mainly responsible for the formation of lecithin in the liver has been suggested by Haines (1966).

For the reasons mentioned above, intravenously injected radioactive Ch was thought to be useful for studying the turnover and compartmentation of ACh in the brain. Consequently it is of importance to know the fate of the injected labelled Ch also in plasma. In the literature there is some evidence that the plasma concentration of Ch is remarkably constant around 10 nmoles/ml and that it is neither changed by exercise or by a meal, nor greatly affected by various liver or kidney diseases (Bligh 1952). Moreover when exogenous Ch is injected into the plasma, the increased Ch level is rapidly corrected to a normal value (Bligh 1953). However a more detailed evaluation of the time course of the metabolic disposal of Ch when administrating high doses or tracer amounts of Ch has not previously been made. As seen in paper I, doses of 500 and 2,000 nmoles given intravenously produced in 2 sec a marked increase of the Ch plasma level (147 and 581 nmoles/ml). Despite a negligible urinary excretion of radioactivity the plasma concentration was normal (10–15 nmoles/ml) after 5 and 20 min respectively. After injection of 10 nmoles Ch no increase in plasma Ch was observed and the biological half-life in plasma was about 5 min.

Most of the injected radioactive Ch was recovered as PhCh in tissues (jejunum, liver and brain). The responsible enzyme (Ch kinase) has been found in the brain (highest activity), stomach, kidney intestinal mucosa, liver and heart (McCaman 1962). Apparently most organs of the mouse seem to be able to convert Ch to PhCh. The latter can be further metabolized in the organs, via the Kennedy pathway to lecithin (Fig. 1).

From findings in paper I it seems evident that the oxidation of Ch to betaine in most tissues is a minor pathway in comparison with the phosphorylation of Ch. This is contrary to suggestions made by Bligh (1953) who proposed that the Ch oxidation was the major pathway. The importance of the oxidation step is apparently to generate methyl groups, as betaine is a better methyl donor than Ch (Borsook and Dubnoff 1947). However, betaine is one of the major metabolites in plasma after (^{3}H)-Ch injection. This betaine must be carried to the plasma from tissues as plasma is not able to metabolize Ch *in vitro* (Sparf, unpublished results). On the other hand, no labelled PhCh is found in the plasma which is the major metabolite in most tissue. This is due to hydrolysis of PhCh by enzymes present in plasma, which was observed after intravenous injection of labelled PhCh (I). Labelled GPhCh or CDPCh could not be found in the plasma after intravenous injection of labelled Ch. The appearance of labelled phosphatidylcholine in plasma was very slow (I).

3.2. The source of free choline in the brain

Experiments with the perfused cervical ganglion in the rat have shown that the Ch concentration in the perfusion medium is of great importance for optimal ACh synthesis during synaptic activity (Birks and MacIntosh 1961, Collier and MacIntosh 1969). It also appears that about half the Ch formed from released ACh is immediately recaptured and resynthetized into ACh (Collier and MacIntosh 1969).

In general, investigations have shown that Ch is transported to the tissues by a low affinity uptake mechanism, e.g. kidney (Rennik 1958, Sung and Johnstone 1965, 1969), giant axons of Loligo (Hodgkin and Martin 1965), erythrocytes (Martin 1968), brain cortex slices (Schuberth *et al.* 1966 a, Schuberth, Sundwall and Sörbo 1967), synaptosomes (Marchbanks 1968 b, Potter 1968, Diamond and Kennedy 1969, Abdel Latif and Smith 1972) and microsomal, synaptosomal, mitochondrial and synaptic vesicle fractions of rat brain (Diamond and Milfay 1972).

Is there a similar transport mechanism of free Ch from plasma into brain or is Ch first converted into a more lipid soluble form. The latter alternative has been suggested by Ansell and Spanner (1971). The postulated mechanism is based on the finding that from one to six hours after intraperitoneal injection of labelled Ch, ethanolamine, dimethylaminoethanol or methionine, lipids in the liver and brain became radioactive "whereas no labelled Ch was detectable in the blood or the brain". However, the biological half lives of radioactive Ch in the plasma and the

brain vary between 5 and 10 min (I). Therefore, in the time interval (0—60 min) incorporation of labelled free Ch in the plasma into the brain lipids could have occurred. Thus, in view of the long interval (1 and 6 hours) in the above experiment, it is not justified to consider lipid Ch in plasma as the main precursor of brain Ch. Another indication that Ch is not mainly transported to the brain as lipid-Ch is that, the fatty acid composition of brain and liver phosphatidylcholine is quite different (Ansell and Spanner 1971). Moreover their demonstration of a precursor product relationship between CDPCh and phosphatidylcholine after *intra-cerebral* injection of labelled Ch speaks against phosphatidylcholine in plasma as the main Ch source for brain. If phosphatidylcholine is transported to a substantial extent from the plasma to the brain, then this would result in a lower SA of phosphatidylcholine compared with that of CDPCh.

Ch liberated from PhCh or phospholipid-Ch, does not seem to be an important source of Ch for ACh synthesis under physiological conditions in perfused superior cervical ganglion of the rat (Collier and Lang 1969). On the contrary as previously mentioned, free Ch must be supplied to the perfusion medium for optimal ACh synthesis.

The first evidence, for free Ch in plasma being a precursor of brain Ch, was shown in experiments in which methyl labelled Ch was injected intravenously into mice (III). As soon as 15 sec after the injection, about 15 % of the radioactive Ch, captured by the brains of conscious mice, are in the form of ACh. The SA time curve for Ch in the brain rapidly crosses that of ACh at a point where the SA of ACh was maximal, which suggests a direct precursor-product relationship (Fig. 3). The only radioactive, acid-soluble Ch-metabolites present during the time interval 0.25—20 min are Ch, ACh and PhCh (I). It seems improbable that within this short time interval the Ch has been converted to phospholipid Ch before entering the brain. The previously described time course of the metabolite pattern in the plasma and brain after intravenous injection of labelled Ch (I) is another evidence in favour of plasma Ch as the immediate precursor of brain Ch.

By injecting two different doses of Ch containing the same amount of radioactivity it was demonstrated that the relative amount of labelled Ch incorporated in the brain is decreased slightly dose dependent, if a non-tracer dose (750 nmoles) of radioactive Ch was injected instead of a tracer dose (10 nmoles). Moreover incorporation of Ch into the brain, after a 3 minute infusion, was three times more effective than after a pulse injection of the same tracer dose, as can be seen in Table II. From these findings it seems probable that there is a transport of free Ch from the plasma to the brain, which is mediated by a carrier which is saturated already at physiological-plasma Ch levels (I).

Diamond (1971) obtained evidence in support of a carrier mediated transport

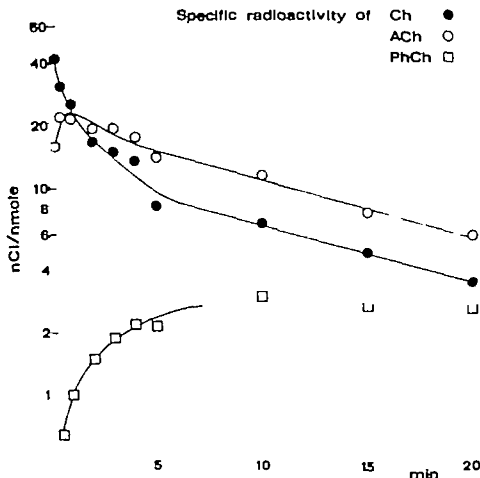


Fig. 3 The specific radioactivities of choline, acetylcholine and phosphorylcholine in the brain at different times following intravenous injection of 160 µCi (10 nmoles) (H)-choline. Endogenous values used Ch 40 nmoles/g; ACh 15 nmoles/g (ref. See text); PhCh. 380 nmoles/g (Ansell and Spanner). Each point expresses the mean of 3–8 experiments.

of Ch from the plasma to the brain, which is saturated at Ch concentrations ten times the normal serum levels. This difference in saturation level may be explained by the findings of Yamamura and Snyder (1972). They found that synaptosomes isolated from rat brain accumulate Ch by two kinetically distinct processes, a high affinity uptake system (K_m=10 M) and a low affinity system (K_m=10 M). The high affinity uptake system was associated with a consider

Table II

A comparison of the transport into brain of radioactive Ch following an intravenous pulse injection and a 3 min infusion. 160 µCi (^{3}H)–Ch (10 nmoles) were administered at zero time to mice by injection or infusion. The animals were killed 1, 3 and 5 min after the injection and 3 min after the start of the infusion. The values of the radioactive Ch and its metabolites are expressed as the mean ±S.E., n = the number of experiments.

Rout of administration	Time min	n	(^{3}H)–tot.	(^{3}H)–PhCh	(^{3}H)–Ch	(^{3}H)–ACh
I.v injection	1	3	29.9 ± 1.2	7.0 ± 0.4	15 7 ± 1.6	4.5 ± 0.5
	3	5	34.5 ± 1.6	16.8 ± 0.9	13.2 ± 1.5	6.5 ± 0.4
	5	3	31.1 ± 1.2	19 7 ± 2.9	9 6 ± 0.2	5.5 ± 0.2
Infusion	3	6	75.3 ± 10.6	14.3 ± 1 4	38.2 ± 6.3	8.3 ± 0.8

able formation of ACh, and was assumed to represent selective Ch accumulation by cholinergic neurons. This indicates that the low-affinity uptake system was studied by Diamond as only non-tracer doses of Ch were used at the intravenous injection.

Recently a third hypothesis concerning the origin of the brain has been published (Dross and Kewitz 1972). They suppose that phosphorylated derivates of ethanolamine (phosphorylethanolamine, CDP-ethanolamine) can be methylated in the brain to form the respective Ch derivatives. These new aspects of the origin of Ch in the brain are not compatible with the generally held view (see above) that the brain cannot synthetize Ch *de novo* The hypothesis as advanced by Dross and Kewitz (1972) is based on the following findings

i) The concentration of free Ch in the brain increases at a velocity of about 20 nmoles/g and min immediately after decapitation. This is assumed to be due to the cessation of venous drainage while normal Ch synthesis proceeds.

ii) An arteriovenous difference was observed between the Ch concentration of the femoral artery (12±0.5 nmoles/ml) and that of the jugular vein (19 4±1 7 nmoles/ml).

iii) After intravenous injection of (^{14}C)-Ch the peak of the SA of Ch in the brain is reached in less than 1 min but amounts to only one tenth of the SA of Ch in plasma. There is a parallel decline of the SA in both compartments.

From these findings they conclude that Ch readily penetrates the blood-brain barrier and that the concentration gradient is directed from the brain to the venous blood. Ch is continuously formed in the brain, which process continuously

dilutes the labelled Ch, and prevents the SA in the blood and the brain from becoming equal. An alternative explanation would be that there is a more rapid synthesis of ACh and PhCh from labelled Ch in the brain compared with the transport rate of labelled Ch from the plasma to the brain. If this is the case, one of the assumptions, previously mentioned, for estimating the turnover of ACh by measuring the initial formation of labelled ACh in the brain, is probably not ful filled. As a consequence, this would result in a too low value of the turnover

After intravenous injection of labelled Ch the SA of PhCh in the brain did not reach the SA of Ch (Fig. 3 Dross and Kewitz 1972) Although Ch is certainly a first order precursor of PhCh the finding indicates that PhCh reaches the pool also via an alternative pathway e.g., phosphorylation of ethanolamine or hydrolysis of compounds containing PhCh. The lower SA was used by Dross and Kewitz as an additional argument in support of the existence in the brain of a methylation pathway However in metabolic systems in which the product compartment is not homogeneous, the SA curves of precursor and product will necessarily not cross (Zilversmit 1960). This might occur in the present case, as a synthesis of labelled ACh proceeds simultaneously with a synthesis of labelled PhCh.

In short, the conclusion of the present author is that, free Ch seems to be transported to the brain from the plasma via a carrier-mediated, transport mechanism which is saturated at physiological plasma levels of Ch (I). This process is connected with a rapid synthesis of ACh in the brain. It seems as if a minor part of the brain Ch may be the result of a lipid-Ch transport, as an arterio-venous difference of Ch has been demonstrated There is no direct evidence yet that Ch can be synthetized *de novo* in the brain.

4 TURNOVER OF ACETYLCHOLINE IN THE BRAIN AND ITS NERVE ENDINGS

4.1. Estimation of turnover rates of acetylcholine

The rate of formation and the rate of disappearance of the radioactive ACh formed in the brain from intravenously injected labelled Ch, have been used to determine the turnover rate of ACh in the brain. By measuring both the initial rate of formation of labelled ACh and the isotopic dilution of the labelled Ch in the brain the turnover rate was estimated at 50 nmoles/g brain/min (III). This calculation was based on a 40 % conversion of labelled Ch to ACh per g of brain and min, and on an endogenous Ch value of 115 nmoles/g (III). However as discussed earlier a Ch content of about 50 nmoles/g brain is more probable, which would decrease the calculated turnover rate to about 20 nmoles/g/min.

The turnover study in paper III can be criticized since it was performed with a dose of (^{3}H)-Ch of 500 nmoles. However as seen in Table III very similar results were obtained with a dose of 10 nmoles of Ch which leaves the plasma level completely unchanged. When the timecourse of the SA of Ch and ACh in the whole brain is followed, a precursor product relationship could be rather well established, as previously mentioned (Fig. 3). Objections can be raised against the calculation of turnover rates by the present method, as the estimation is based on the assumptions that the brain Ch is localized in one pool, and that the injected labelled Ch is rapidly equilibrated with the endogenous Ch pool. How valid are these assumptions?

The ratio labelled Ch:ACh was rather similar (about 2 1) in both the whole

Table III
The conversion of (^{3}H)–Ch to (^{3}H)–ACh in the brain after a pulse injection intravenously of 500 nmoles of (^{3}H)–Ch and 10 nmoles of (^{3}H)–Ch.
The values are expressed as the mean of ((^{3}H)–ACh as % of (^{3}H)–Ch) ± S.E., n number of experiments.

Time (min)	(^{3}H)–Ch (500 nmoles) (^{3}H)–ACh/(^{3}H)–Ch(%)	n	(^{3}H)–Ch (10 nmoles) (^{3}H)–ACh/(^{3}H)–Ch(%)	n
0.25	10.4 ± 1.32	4	13 4 ± 0.98	6
0.50	15 1 ± 1.15	5	24.9 ± 0.83	7
1	24.2 ± 0.10	2	30.0 ± 2.00	8
2	37 4 ± 0.72	2	41.0 ± 2.01	3
5	38.9 ± 0.45	2	59 6 ± 2.49	4
10	46.1 ± 1.05	2	63 3 ± 3.28	4

brain and the isolated nerve endings during the time interval 2—20 min after intravenous injection of Ch (IV). The half-lives of both Ch and ACh were also approximately the same (7 min) in both the whole brain and the nerve endings (IV). Since radioactive and endogenous ACh is almost exclusively localized in the nerve endings, the findings indicate that also radioactive Ch is mainly localized in the nerve endings. This means that the assumption mentioned in the general approach concerning a model of an open single compartment system for the calculation of turnover can be used. The fact that the ratios mentioned become equal already after 2 min points to a rather rapid mixing of radioactivity with the endogenous pools.

A further support for an immediate, main transport of Ch in the plasma to the nerve endings is the previously mentioned, high affinity uptake of Ch by the synaptosomes of the brain *in vitro* which is associated with considerable ACh synthesis (Yamamura and Snyder 1972). The rather similar half life of Ch in plasma and Ch and ACh in the brain may also be an indication of a rapid equilibration of plasma Ch with brain Ch. The existence of a specific Ch uptake by cholinergic nerve endings is indicated by experiments performed by Kuhar *et al.* (1973) They found that the Ch uptake at 10^{-5} to 10^{-4}M by synaptosomes *in vitro* was reduced by lesions only in brain regions in which a high cholinergic input is probable. These results also speak in favour of the hypothesis that cholinergic nerve endings are responsible for the bulk of the free Ch accumulated by the brain tissue.

Since mouse brain contains about 15 nmoles/g of ACh (13 nmoles/g, Crossland and Merrich 1954 16 nmoles/g, Consolo *et al.* 1972) the estimated turnover rate would mean that the brain renews its ACh store about every minute. This rate lies between the turnover rate found in the perfused cervical ganglion (10 nmoles/g/ min) and the initial restitution rate of ACh in the brain after partial depletion of the ACh store by electro-shock (40 nmoles/g/min) (MacIntosh 1963 Richter and Crossland 1949). The latter value is rather near the maximal synthesis rate obtainable *in vitro* by ChAc from 1 g mouse brain tissue (60 nmoles/g/min) (Schrier and Shuster 1967).

Another approach to calculate the ACh turnover in the brain is also possible. *The size of the Ch pool that is used for ACh synthesis can be calculated by using* the ratio ? 1 between the labelled Ch and the labelled ACh in the nerve endings 2 to 20 min after intravenous injection of labelled Ch (IV). If about 80 % of the endogenous ACh in mouse brain (15 nmoles/g) are in the nerve endings (Hebb and Whittaker 1958), and 50 % of the ACh in the nerve endings are located in the cytoplasm (Chakrin and Whittaker 1969) the Ch concentration in the synaptosomal cytoplasm (compartment for ACh synthesis?), calculated on the

ratio 2 1 between labelled Ch and ACh, would correspond to about 12 nmoles/g brain. However this calculation according to pool size was based on the assumption that the incorporation of ACh into the synaptic vesicles is very slow (Chakrin and Whittaker 1969 Marchbanks 1969). This means that most of the radioactive ACh formed during this short time interval is in the cytosol compartment. However as shown in paper V there is probably a very rapid incorporation of radioactive ACh into a high molecular weight fraction of the nerve endings. As will be discussed in the next paragraph it also seems as if it might be a very intimate coupling, between the synthesis and storage of ACh, to a high molecular weight constituent in the nerve ending. This would indicate that the ACh pool in the nerve ending may still be regarded as an open single compartment and then consists of up to 12 nmoles ACh/g. From the ratio labelled Ch labelled ACh (2.1) the Ch pool could be estimated at 25 nmoles/g. The turnover rate of ACh could then be calculated at about 10 nmoles/g/min (40 % initial conversion of labelled Ch to labelled ACh/g/min).

A third method which is a more speculative way to calculate the turnover was used in paper IV Based on calculations of the volume of the nerve endings (Marchbanks 1968 b) and kinetic data of ChAc (Schuberth 1966) the rate of ACh formation at the estimated concentrations of the substrates was calculated at about 10 nmoles ACh/g/min (IV). Since the optimal synthesis rate is 60 nmoles ACh/min obtainable *in vitro* by ChAc from 1 g mouse brain (Schrier and Shuster 1967) instead of 160 nmoles/min, which was erroneously cited in paper IV this value is probably about 5 nmoles ACh/g/min.

An often used way to estimate the turnover of catecholamines is to study the decay curves of the radioactive labelled transmitter after blocking its synthesis. The disappearance curve of labelled ACh from the brain may reflect not only the turnover of ACh but also other factors such as a continuing synthesis from the remaining labelled Ch, and the re-uptake to the nerve endings of Ch from released and hydrolyzed, labelled ACh. In ganglia half the Ch formed from released ACh seems to be recaptured and resynthetized to ACh (Collier and MacIntosh 1969). The ACh turnover (~ 1 nmole/g/min) calculated from the half-life (t 1/2 ~ 7 min) of labelled ACh is about twenty times lower than the turnover estimated from the initial formation of labelled ACh (20 nmoles/g/min). The half-lives of labelled Ch and ACh in the brain may also be influenced by the Ch half-life in plasma. There is about the same half-life of free Ch in the plasma and the brain (I)

It is not possible to calculate the synthesis rate from the changes in the SA of the precursor and the product in the plasma and the brain when (^{3}H)-Ch was pulse injected. This is owing to the SA of Ch in plasma being higher than that of the brain during the whole time interval studied. The latter approach has been used for

determining the synthesis rate of dopamine, noradrenaline and serotonin in the brain stem (Neff *et al* 1971).

However by infusing (^{3}H)-Ch into rats and following the SA of Ch and ACh in the brain, and assuming an open single compartment model, the efflux rates of ACh have recently been determined in the cortex, midbrain and brainstem (Saelens *et al* 1973). In the cortex the rate was 20 nmoles/min/g, which is in good agreement with the turnover rate found in the whole brain of the mouse when applying our technique, and confirmed by Saelens *et al* 1973 who used the same method.

A valuable pharmacological tool, when studying the ACh turnover by means of the declining ACh curve, would be a centrally active ChAc inhibitor. Unfortunately the inhibitors available today are unsuitable for this purpose (Aquilonius *et al* 1971) as has been previously mentioned.

From the above discussion, it is obvious that a number of assumptions must be made in order to be able to calculate the turnover of ACh in the brain. It has not been possible to ascertain to which extent these assumptions are valid. However by using two different ways to calculate the ACh turnover rate, rather similar values were obtained 10–20 nmoles ACh/g/min).

4.2 Compartmentation of newly synthetized acetylcholine

As previously mentioned endogenous ACh in the nerve endings is distributed in at least two morphological compartments, namely in the cytoplasm and in the vesicles (de Robertis *et al* 1963 Whittaker *et al* 1964). The incorporation of radioactive ACh into different pools of the nerve endings has been studied by Chakrin and Whittaker (1969) by injecting radioactive Ch intracortically into anaesthetized cats and guinea pigs. These experiments showed a slow incorporation of the synthetized transmitter into the vesicles as compared with the cytosol. Similar results were obtained when the conversion of radioactive Ch to ACh was studied in the nerve endings of the brain *in vitro* (Marchbanks 1968b Richter and Marchbanks 19 1). Indirect evidence of an additional small ACh pool with a high turnover was obtained when Ch was injected intraventricularly into conscious guinea pigs under local anaesthesia (Barker *et al* 1970). These results were obtained by a comparison of the SA of synaptosomal ACh at different stages of the isolation procedures. Thus the pool was readily lost when synaptosomes were separated, on a sucrose density gradient, from the other constituents of the crude mitochondrial fraction (P_2 in Fig. 4), myelin and mitochondria.

Intracortical injections seem rather unphysiological especially since in the

PREPARATION OF A HIGH (HMWF) AND LOW (LMWF) MOLECULAR WEIGHT FRACTION FROM BRAIN NERVE ENDINGS

Homogenization of a mouse brain in 0.3 M sucrose containing 10^{-4} M eserine

Centrifugation at 1000 g for 10 min

Pellet (P 1) (nuclei, myelin, celldebris)

Supernatant (S 1)

Centrifugation at 20000 g for 15 min

Pellet (P 2) (synaptosomes, mitochondria, myelin)

Supernatant (S 2)

Suspended in 0.6 ml ice-chilled 10^{-4} M eserine left for 15 min

Centrifugation at 25000 g for 15 min

Supernatant (S 3)

Pellet (P 3) (mitochondria, synaptosomal membranes)

Drained through Sephadex G-25 column

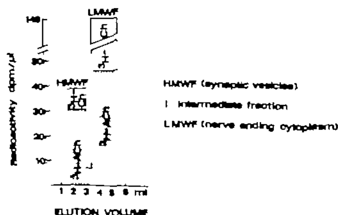


Fig. 4 Subcellular fractionation of a high (HMWF) and a low (LMWF) molecular weight fraction from a mouse brain. The mouse was injected intravenously with 566 µCi (H)-Ch and killed by blow on the neck three min later. The homogenisation of the brain was started within 30–45 sec after death.

present study there are indications, that plasma Ch is the physiological precursor of brain ACh. Therefore, the incorporation of labelled ACh into the different pools of the nerve endings was studied by using the present *in vivo* technique. The content of lysed synaptosomes was separated into a high (HMWF) and a low (LMWF) molecular weight fraction by a modification of the current gel filtration procedures (Marchbanks 1968 b Kadota and Kanaseki 1969).

In our experiments the lysed, nerve ending fraction was first centrifuged, and mitochondria and membrane fragments were pelleted before passing the supernatant through a Sephadex G 25 fine column. By eluting the column with 10 mM of sodium chloride two distinct fractions could be obtained. The whole isolation procedure of the HMWF and the LMWF which, in this way is comparatively rapidly performed, is shown in Fig. 4

The intention was to follow the SA of both ACh and Ch in these two fractions, in order to elucidate the compartmentation of ACh and the site of its biosynthesis in the nerve ending. After intravenous injection the incorporation of radioactive ACh into both the LMWF and HMWF is very rapid (V) Already 15 30 sec after the injection, the incorporation of both labelled Ch and ACh were maximal in the two fractions. An interesting finding is that the ratio of (^{3}H)-ACh/(^{3}H)-Ch was about 10 times higher in the HMWF than in the LMWF

When the SA of ACh was calculated, a higher SA of ACh was found in the HMWF than in the LMWF contrary to the findings of Chakrin and Whittaker (1969) who reported that the cytoplasmic fraction of ACh showed a SA which was twice that of the vesicular However the estimations were done only at one time interval and, as late as 60 min after an intracerebral injection of labelled Ch. Furthermore the animals were anaesthetized with a barbiturate, which decreases the ACh turnover in the brain (III).

Our study (V) demonstrated that the SA of ACh was significantly higher in the HMWF in 18 of 21 experiments, in the interval 0.25–15 min after the intravenous injection of labelled Ch. In two of the three experiments where the SA of ACh in the LMWF was higher than in the HMWF this was observed at 15 min. Owing to the rapid incorporation of labelled Ch and ACh, no distinct maximum could be established at the times studied. Therefore, it was not possible to establish a precursor product relationship from the kinetics of the SA of ACh in the HMWF and the LMWF

The SA of ACh in the two fractions is not known in the time interval 0–0.25 min but two possibilities exist

i) the SA of ACh is higher in the HMWF than in the LMWF also during this period In that case synthesis

a) proceeds independently in the two compartments but at a higher rate in HMWF. In fact a more rapid half life of the SA of ACh in the HMWF (t 1/2 ~ 7 min) compared with that of the LMWF (t 1/2 ~ 12 min) is indicated in the time interval 1–15 min after the (^{3}H)-Ch injection.

b) Alternatively synthesis occurs only in the HMWF but a leak of labelled ACh from HMWF to LMWF occurs during the isolation procedures.

c) If the product compartment (in this case ACh in the LMWF is not homogeneous, the two SA curves will not cross each other (Zilversmit 1960). The latter explanation is likely if the ACh synthesis occurs in the HMWF but the LMWF consists of a two- or multi-compartment system.

ii) The SA of ACh in the LMWF crosses that of the HMWF at an extremely early stage, indicating a primary synthesis in the LMWF.

To further elucidate the site of the ACh biosynthesis, the SA of Ch were estimated in the two fractions. However immediately after homogenization, a value of 185 nmoles Ch/g was found. On the assumption that the yield of the endogenous Ch is the same as that of the labelled Ch in the P_2 fraction i.e. 11 % (V), the Ch concentration in this fraction should be about 5 nmoles/g. The actual value found was, however 41 nmoles/g. Therefore, the concentration found in the lysed P_2 is probably too high. No increase in the amount of Ch occurs during chromatography of the lysed P_2 on the Sephadex column. Incubation experiments indicate that the Ch concentration in the HMWF remains unchanged during the fractionation procedure (V). Consequently the Ch concentration found in this fraction may correspond to *in vivo* conditions. The considerably lower SA of Ch, than that of ACh in the HMWF at all the times investigated, indicates that ACh is probably not synthetized from the Ch within the HMWF. On the other hand, the concentration of Ch in the LMWF *in vivo* may possibly be about seven times lower (5 nmoles/g instead of 36 nmoles/g) than indicated in paper V.

If this is true the SA of the Ch in the LMWF (presumably corresponding to cytoplasmic Ch) would, at least at the shortest time-intervals, be higher than that of the ACh in the HMWF (presumably corresponding to vesicular ACh). For these reasons cytoplasmic Ch is the most likely immediate precursor of vesicular ACh. A possible hypothesis in explanation of the findings is that, the ACh biosynthesis occurs in the cytoplasmic compartment, but in close association with synaptic vesicles or another high molecular constituent. Using an *in vitro* system, Ritchie and Goldberg (1970) were able to demonstrate an incorporation of ACh into a vesicle fraction only in the presence of concomitant ACh synthesis. Conse-

quently a direct coupling seems plausible between the biosynthesis and the binding of ACh to a constituent present in the vesicular fraction.

This hypothesis will also explain the failure to demonstrate an uptake of ACh by isolated vesicles *in vitro* (Marchbanks 1968 a) and the experiments by Katz, Salehmoghaddam and Collier (1973) which showed that ACh taken up by nerve endings cannot be released by electrical stimulation of the nerve. It has also been demonstrated that the amount of exogenous ACh accumulated by a cervical ganglion is small when compared with the amount of ACh synthetized from exogenous Ch (Collier and MacIntosh 1969).

A puzzling finding in our study (V) was the low yield of (^{3}H) ACh in the lysed fraction P_3 (28 %) as compared with the corresponding recovery of endogenous ACh (60 %). A somewhat higher yield of labelled ACh (37—41 %) in the isolated P_3 fraction was obtained in a former study (IV). A still higher yield of endogenous ACh in the P_3 fraction of 70 % was found by Whittaker (1959).

This unexpected discrepancy between the recovery of labelled and of endogenous ACh could be due to a more labile form of binding of newly synthetized ACh than of preformed ACh. This interpretation is also in agreement with results obtained by Barker *et al* 1970.

The discrepancy could be explained by populations of nerve endings from some regions of the brain with a high ACh turnover resulting in a large proportion of newly synthetized transmitter or by a heterogeneous vesicle population in the nerve ending. After labelling the ACh store under physiological conditions, the functionally most active vesicles, which are presumably close to the presynaptic membrane may incorporate ACh of a higher SA than those vesicles which are more centrally located and probably more inactive. The latter hypothesis of heterogeneous vesicles is supported by a report from Roth *et al* 1968 who isolated two populations of vesicles containing norepinephrine. ACh containing vesicles with different characteristics have also been isolated from guinea pig brain (Kadota and Kanaseki 1969).

To further elucidate the compartmentation of newly synthetized ACh in different nerve ending and vesicle populations, experiments for studying ACh turnover in different brain regions and vesicle fractions are now in progress.

5 EFFECT OF DRUGS ON THE TURNOVER OF ACETYLCHOLINE

As mentioned in the introduction the amount of ACh in the brain changes when the functional activity of the organ is altered by certain drugs. For example, the administration of pentobarbital and oxotremorine is associated with an increase in the ACh content whereas administration of e.g., atropine is associated with a decrease (Giarman and Pepeu 1962, Holmstedt, Lundgren and Sundwall 1963 Consolo *et al.* 1972). Further it has been demonstrated that barbiturates decrease and atropine increases the release of ACh from the cerebral cortex (Mitchel 1960 Bartolini and Pepeu 1967). However by studying the effect of these drugs on the ACh turnover in the brain more knowledge should be obtained concerning the regulation of the synthesis, storage and release of ACh. Consequently the effects of a barbiturate (pentobarbital), a cholinergic (oxotremorine), and an anticholinergic drug (atropine) on the turnover have now been studied by means of the present *in vivo* techniques.

When the effect of pentobarbital on the initial rate of formation of labelled ACh was studied, it was found that the turnover rate was diminished to 1/4 of its normal value (III). As the drug causes a reduction in the body temperature of mice, this has also been compensated for by keeping the mice at 38°C As shown in Fig. 5 the reduced rate of synthesis of radioactive ACh is due, only to a minor degree, to the hypothermia (Schuberth, Sparf and Sundwall 1970 b).

During anaesthesia the radioactive Ch in the brain was found to be about 10–20 % higher than in conscious mice. Hence, one could suspect that the reduced rate of ACh synthesis reflects an overall decrease in Ch metabolism. Therefore, experiments were performed in which the major products of Ch metabolism in the brain were measured. It was observed that the biosynthesis of both the labelled PhCh and the phospholipids was much less affected than that of ACh (Fig. 6). This indicates that the effect of pentobarbital on ACh synthesis is rather specific. This specificity was also found to be dose related (Fig. 7) (Schuberth, Sparf and Sundwall, to be published).

Oxotremorine, at an intraperitoneal dose of 0 5 mg/kg, which was found to be the threshold dose for tremor also diminished the ACh turnover rate in the brain to 1/4 (III). The effect occurred even when the decrease in the body temperature of the mice was inhibited (Fig. 8). This effect on turnover may be compared with earlier findings which showed that the increased ACh levels in *rat brain* produced by oxotremorine (Holmstedt, Lundgren and Sundwall 1967), are not mediated by a fall in body temperature (Campbell, Hamn and Jenden 1969). Oxotremorine also had a dose dependent effect on the ACh turnover Uptake of radioactivity

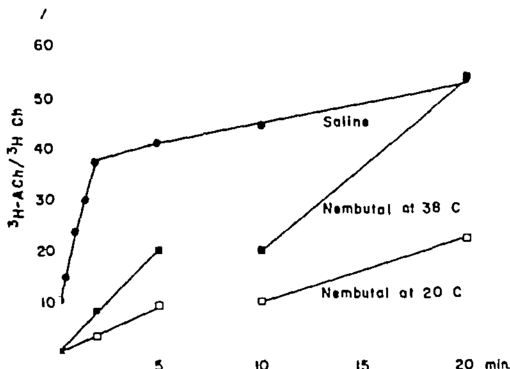


Fig. 5 Synthesis of radioactive ACh in mouse brain *in vivo*. 100 µCi (³H) Ch (500 nmoles) were given intravenously at zero time, and the ratio, radioactive ACh/radioactive Ch, was analyzed at different times. The mice were killed by immersion in liquid nitrogen. Pentobarbital (60 mg/kg) was injected intraperitoneally 30 min before the injection of (³H) Ch. In one group of the pentobarbital treated mice, hypothermia was prevented by warming the animal at 38°C. Each point represents the mean of 4 experiments

and incorporation of Ch into PhCh and phospholipids were only slightly affected (Fig. 9). Contrary to the findings in the present investigation *in vivo*, oxotremorine increases the ACh synthesis in cerebral cortex slices *in vitro* (Howes *et al.* 1970). The explanation for these different results, which were obtained *in vitro* and *in vivo*, is not known.

Oxotremorine and pentobarbital increase the ACh level and decrease the ACh turnover *in vivo*. However probably different mechanisms are involved. This is indicated by the increase in the Ch levels produced by oxotremorine, contrary to pentobarbital (III and Consolo *et al.* 1972).

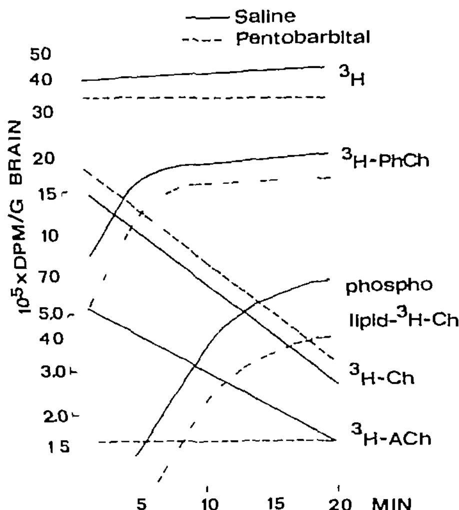


Fig. 6. Effect of pentobarbital on the uptake and metabolism of (^{3}H)-Ch in mouse brain *in vivo* ((^{3}H)—total radioactivity). 160 μCi (^{3}H)-Ch (10 nmoles) were injected intravenously 30 min after intraperitoneal injection *of pentobarbital (60 mg/kg)*. The mice were killed in liquid nitrogen at different times after the injection of (^{3}H)-Ch. Hypothermia was prevented by warming the mice at 38°C. Each point represents the mean of 4 experiments.

Atropine at an intravenous dose of 5 mg/kg had no measurable effect on the initial rate of ACh formation (VI). Therefore its possible effect on the exponential decline of (^{3}H)-ACh in the brain was investigated. Atropine was injected intra

FATE OF ^{3}H-CHOLINE IN MOUSE BRAIN

Pentobarbital

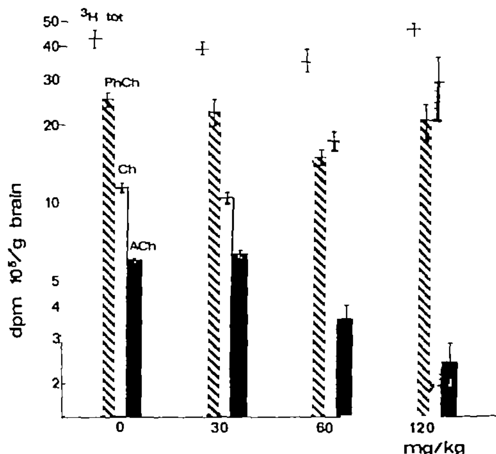


Fig. 7 The effect of different doses of pentobarbital on the uptake and metabolism of (^{3}H) Ch in mouse brain *in vivo*. (H)-tot. = total radioactivity 160 µCi (H)-Ch (10 nmoles) were injected intravenously 30 min after intraperitoneal injection of pentobarbital. The mice were killed in liquid nitrogen 5 min after the injection of (^{3}H)-Ch. Mean ± S.E. is indicated, n=2–3.

venously 3 min after the injection of labelled Ch, when the labelled ACh had reached its peak value in the brain It was observed that atropine decreased the half-life of labelled ACh to ~ 5 min compared with a half-life of ~ 10 min in the control group (VI). The SA of ACh did not change 2–17 min after the atropine injection. No significant change in the time curves of labelled Ch, PhCh or total radioactivity occurred.

The increase in the ACh turnover indicated by the more rapid half-life of (^{3}H)-

Relationship between ^{3}H-ACh and ^{3}H-Ch in mouse brain at different times after injection of ^{3}H-Ch

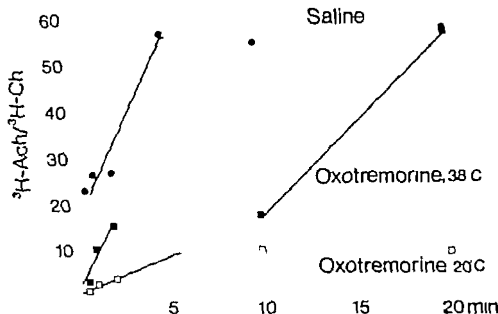


Fig. 8. Synthesis of radioactive ACh in mouse brain *in vivo*. 160 µCi (^{3}H) Ch (10 nmoles) were given intravenously at zero time, and the ratio radioactive ACh/radioactive Ch in whole brain was analyzed at different times. Oxotremorine (1 mg/kg) was given intraperitoneally 30 min before the injection of (^{3}H) Ch. Each point represents the mean of 2 experiments.

ACh in the brain probably reflects a higher release of ACh from the nerve endings. Many types of experiments have indicated, that it is most recently stored or synthetized transmitter which is preferentially released (superior cervical ganglion, Collier 1969 diaphragm, Potter 1970 splenic nerve, Kopin *et al.* 1968 caudate nucleus, Benson *et al.* 1969). The expeiments in paper VI are not readily consistent with the hypothesis that the most recently synthetized transmitter is the most readily released. This may be explained by the fact that the release of ACh after atropine treatment differs in this respect, e.g. that all ACh pools are equally affected. However more experiments are needed to clarify this.

The effect of pentobarbital was also studied after the injection of (^{3}H)-Ch. When injecting a dose of 40 mg/kg of pentobarbital intravenously 3 min after the injection of labelled Ch, the endogenous ACh had increased about 50 % already 2 min after the pentobarbital injection. At that point of time the mice had lost their

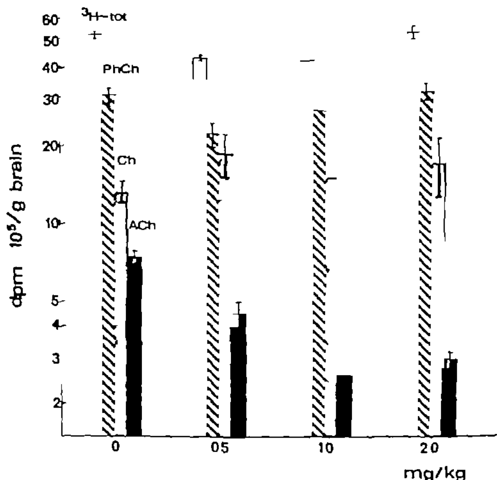


Fig. 9 The effect of different doses of oxotremorine on the uptake and metabolism of (^{3}H) Ch in mouse brain *in vivo*. (H)-tot. = total radioactivity 160 µCi (^{3}H)-Ch (10 nmoles) were injected intravenously *30 min after intraperitoneal injection of oxotremorine. The mice were killed in liquid* nitrogen 5 min after the injection of (H)-Ch. Mean ±S.E. is indicated, n=2.

righting reflex and the reflex was lost throughout the observation period (20 min). As shown in Fig. 10 the half life of (^{3}H)-ACh is increased (t 1/2 ~ 20 min) after injection of pentobarbital, while the curves for (^{3}H)-PhCh and total radioactivity were not affected at all. During preliminary experiments it was found that, contrary to the controls, the SA of ACh is just slightly changed, during the time

FATE OF ^{3}H-CHOLINE IN MOUSE BRAIN

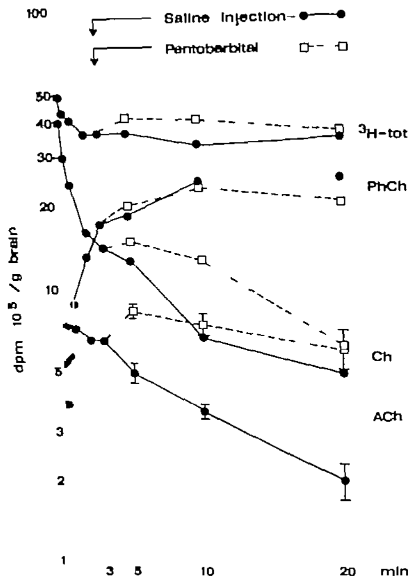


Fig. 10. The effect of pentobarbital on the metabolism of (^{3}H)-Ch in the brain. Pentobarbital (40 mg/kg) or saline were injected intravenously 3 min after intravenous injection of 160 µCi (^{3}H)-Ch (10 nmoles). The values of (^{3}H)-ACh are expressed as mean ±S.E. Each point is the mean of 4–8 experiments. Vertical bars represent ±S.E.

interval studied, following pentobarbital, which, at least superficially regarded, indicates a reduced release of newly synthetized ACh.

A complicating factor when studying the effect of drugs on the ACh turnover in whole brain is the possible regional differences. That such regional effects exist has been demonstrated by Nordberg and Sundwall, 1973. Differences in the effect of drugs on the ACh turnover on the subcellular level must also be taken in consideration.

When the effect of pentobarbital on the compartmentation of ACh in the nerve endings was studied, the most prominent effect was the markedly reduced incorporation of (^{3}H)-Ch into the (^{3}H)-ACh into the HMWF. The incorporation into the LMWF was affected to a much smaller extent. The endogenous ACh in the HMWF was only slightly increased, which agrees with the findings of Kurokawa *et al.* (1963) and of Beani *et al.* (1969). They found an increase in labile bound ACh (cytoplams?) whereas the stable bound ACh (vesicles?) remains relatively unchanged.

Is there any plausible explanation of the decrease in ACh turnover and the increase of endogenous ACh in the LMWF following pentobarbital? An inhibition of ChAc in rat brain by ACh *in vitro* has been demonstrated by Kaita and Goldberg (1969). They found that ACh, at a concentration of 10 mM inhibited its own synthesis by 10 % and that inhibition is progressively increased to 45 % at a concentration of 100 mM ACh. If there is a product inhibition of ChAc also *in vivo* a local increase of the ACh concentration in the low molecular weight compartment could lead to a decrease in the ACh synthesis there. However after injection of pentobarbital the most pronounced effect on synthetized, labelled ACh was in the high molecular weight compartment. The increase in endogenous ACh in the presumed cytoplasmic compartment after pentobarbital indicates that the regulation of the rate of ACh synthesis might occur in the cytoplasmic compartment, and that synthesis is intimately connected with the storage mechanism. The pentobarbital effect indicates also that the binding of ACh to a vesicular constituent may be of direct functional importance.

Another attractive theory is that it may be the concentration of the precursors acetyl-CoA or Ch which regulates the ACh synthesis. That the level of acetyl-CoA is significantly decreased by oxotremorine at a dose of 1 mg/kg but not at 0.5 mg/kg has been shown by Schuberth *et al.* 1966 b. This finding is, however difficult to interpretate as only about 5 % of the amount of acetyl-CoA in the brain takes part in the ACh formation. Atropine (100 mg/kg) and pentobarbital (30 mg/kg) did not change the acetyl-CoA level.

That the Ch concentration can be proposed as a regulatory mechanism of the ACh synthesis is based upon the fact that there is high-affinity uptake of Ch to

synaptosomes, which is connected with a rapid synthesis of ACh. However both the simultaneous decrease in ACh turnover with an increase in Ch level following oxotremorine, and the increase in ACh turnover and unchanged level of Ch following atropine do not support the latter theory

To further elucidate the mechanisms which regulate the synthesis, storage and release of ACh, the effect of drugs on the turnover of ACh in different brain regions and subcellular fractions, will be studied. Such experiments are now in progress.

6. SUMMARY

ACh in the brain is synthetized from acetyl-CoA and Ch. The latter does not seem to be synthetized in the brain *de novo* (Bremer and Greenberg 1961, Ansell and Spanner 1967, 1968). More probably Ch is transported from plasma to the brain as free Ch (I, III), presumably by a carrier mediated mechanism saturated already at physiological plasma levels of Ch (I). However, Ch may also be transported to the brain as lipid-Ch (Ansell and Spanner 1971), which is indicated by an arterio-venous difference between the Ch level in the femoral artery and jugular vein observed by Dross and Kewitz 1972. In the brain Ch seems to be rapidly equilibrated with the Ch in the nerve endings (IV). The transport of free Ch is associated with a rapid synthesis of ACh (III) which has also been shown in synaptosomes *in vitro* when they accumulate Ch by a high affinity uptake mechanism (Yamamura and Snyder 1973). Hence, plasma Ch seems to be the immediate, major precursor of ACh in the brain in addition to the Ch split off from brain lecithin and the re-uptake of Ch from released and hydrolyzed ACh.

The level of free Ch in the brain seems to be in the range 40 ± 20 nmoles/g (II and Eade, Hebb and Mann 1973) about 2–3 times the ACh level. This broad Ch range may be due to too low specificities of some of the present methods, hydrolysis of phospholipids by acidic protein precipitating agents but also post mortal increase of Ch (II and IV).

By using labelled Ch, intravenously injected into mice the turnover rate of ACh in the brain has been calculated at 10–20 nmoles/g/min. Similar results have recently been obtained by Saelens for the rate of ACh efflux in the cortex of rats (20 nmoles/g/min) found by infusion of (^{3}H)-Ch (Saelens *et al.* 1973). Drugs like pentobarbital and oxotremorine decrease the ACh turnover in the brain (III, Schuberth, Sparf and Sundwall 1970 b). Both drugs increase the ACh level in the brain (Giarman and Pepeu 1962, Holmstedt, Lundgren and Sundwall 1963) but pentobarbital, contrary to oxotremorine, does not seem to increase the Ch level or decrease the acetyl-CoA level in the brain (III, Consolo *et al.* 1972, Schuberth *et al.* 1966 b), indicating that different mechanisms are involved.

ACh synthetized from labelled Ch *in vivo*, is very rapidly incorporated into a high molecular weight fraction, isolated from lysed synaptosomes. The pronounced decrease in the incorporation of ACh in the high molecular weight fraction, presumably a vesicular fraction, caused by pentobarbital, indicates that the binding of ACh to a vesicular constituent may be of direct functional importance (V). By comparing the SA of Ch and ACh in the presumed isolated cytoplasmic and the vesicular fraction, it is postulated that the ACh synthesis

occurs in the cytoplasm but is closely associated with the binding of ACh to a constituent, present in the vesicular fraction (V). The hypothesis will also explain the failure to demonstrate an uptake of ACh by isolated vesicles *in vitro* (Marchbanks 1968 a) and the experiments by Katz *et al.* (1973) which showed that ACh taken up by nerve endings cannot be released by electrical stimulation of the nerve.

ACh in the brain is synthetized from acetyl-CoA and Ch. The latter does not seem to be synthetized in the brain *de novo* (Bremer and Greenberg 1961 Ansell and Spanner 1967 1968) More probably Ch is transported from plasma to the brain as free Ch (I, III), presumably by a carrier mediated mechanism saturated already at physiological plasma levels of Ch (I). However Ch may also be transported to the brain as lipid-Ch (Ansell and Spanner 1971), which is indicated by an arterio-venous difference between the Ch level in the femoral artery and jugular vein observed by Dross and Kewitz 1972. In the brain Ch seems to be rapidly equilibrated with the Ch in the nerve endings (IV). The transport of free Ch is associated with a rapid synthesis of ACh (III) which has also been shown in synaptosomes *in vitro* when they accumulate Ch by a high affinity uptake mechanism (Yamamura and Snyder 1973). Hence, plasma Ch seems to be the immediate, major precursor of ACh in the brain in addition to the Ch split off from brain lecithin and the re-uptake of Ch from released and hydrolyzed ACh.

The level of free Ch in the brain seems to be in the range 40 ± 20 nmoles/g (II, and Eade, Hebb and Mann 1973) about 2–3 times the ACh level. This broad Ch range may be due to too low specificities of some of the present methods, hydrolysis of phospholipids by acidic protein precipitating agents but also post mortal increase of Ch (II and IV)

By using labelled Ch, intravenously injected into mice the turnover rate of ACh in the brain has been calculated at 10–20 nmoles/g/min. Similar results have recently been obtained by Saelens for the rate of ACh efflux in the cortex of rats (20 nmoles/g/min) found by infusion of (^{3}H)-Ch (Saelens *et al.* 1973). Drugs like pentobarbital and oxotremorine decrease the ACh turnover in the brain (III Schuberth, Sparf and Sundwall 1970 b) Both drugs increase the ACh level in the brain (Giarman and Pepeu 1962 Holmstedt, Lundgren and Sundwall 1963) but pentobarbital, contrary to oxotremorine, does not seem to increase the Ch level or decrease the acetyl-CoA level in the brain (III Consolo *et al.* 1972 Schuberth *et al.* 1966 b), indicating that different mechanisms are involved.

ACh synthetized from labelled Ch *in vivo*, is very rapidly incorporated into a high molecular weight fraction, isolated from lysed synaptosomes. The pronounced decrease in the incorporation of ACh in the high molecular weight fraction, presumably a vesicular fraction, caused by pentobarbital, indicates that the binding of ACh to a vesicular constituent may be of direct functional importance (V). By comparing the SA of Ch and ACh in the presumed isolated cytoplasmic and the vesicular fraction, it is postulated that the ACh synthesis

REFERENCES

Abdel-Latif, A.A. and J.P. Smith, Studies on choline transport and metabolism in rat brain synaptosomes. *Biochem. Pharmacol.* 1972. *21* 3005–3021

Ansell, G.B. and S. Spanner The metabolism of labelled ethanolamine in the brain of the rat *in vivo*. *J Neurochem.* 1967 *14* 873–885.

Ansell, G.B. and S. Spanner The metabolism of (Me ^{14}C)choline in the brain of the rat *in vivo*. *Biochem. J* 1968 *110* 201–206.

Ansell, G.B. and S. Spanner The origin and turnover of choline in the brain. In: *Drugs and cholinergic mechanisms in the CNS* Eds E. Heilbronn and A. Winter Försvarets Forskningsanstalt, Almqvist and Wiksell, Stockholm. 1970. pp. 143–162.

Ansell, G.B. and S Spanner Studies on the origin of choline in the brain of the rat. *Biochem. J* 1971 *122*. 741–750.

Appleton, H.D B.N La Du, B.B. Levy M.J Steele and B.B. Brodie, A chemical method for the determination of free choline in plasma. *J Biol. Chem.* 1953. *205* 803–813.

Aquilonius, S-M, L. Frankenberg, K E Stensiö and B. Winblad, *In vivo* studies of two choline acetyltransferase inhibitors. *Acta Pharmacol.* (Kbh.) 1971 *30* 129–140.

Barker L.A., M.J Dowdall, W.B. Essman and V.P Whittaker The compartmentation of acetylcholine in cholinergic nerve terminals. In: *Drugs and cholinergic mechanisms in the CNS* Eds: E. Heilbronn and A. Winter Försvarets Forskningsanstalt, Almqvist and Wiksell, Stockholm. 1970. pp. 193–223

Bartolini, A. and G.C. Pepeu, Investigations into the acetylcholine output from the cerebral cortex of the cat in the presence of hyoscine. *Br J. Pharmac. Chemother* 1967 *31* 66–73.

Beani, L., C. Bianchi, P Megazzini, L. Ballotti and G Bernardi, Drug induced changes in free, labile and stable acetylcholine of guinea-pig brain. *Biochem. Pharmacol.* 1969. *18* 1315–1324

Besson, M.J A. Cheramy P Feltz and J Glowinski, Release of newly synthesized dopamine from dopamine-containing terminals in the striatum of the rat. *Proc. nat. Acad. Sci.* 1969 *62*. 741–748.

Birks, R.I. and F C. MacIntosh, Acetylcholine metabolism of a sympathetic ganglion. *Can. J Biochem. Physiol.* 1961 *39* 787–827

Björnstad, P and J Bremer *In vivo* studies on pathways for the biosynthesis of lecithin in the rat. *J Lipid Res.* 1966. *7* 38–45.

Bligh, J., The level of free choline in plasma *J Physiol.* 1952. *117* 234–240.

Bligh, J The role of the liver and the kidneys in the maintenance of the level of free choline in plasma. *J Physiol.* 1953 *120* 53–62.

Borsook, H. and J W Dubnoff Methionine formation by transmethylation *in vitro*. *J Biol. Chem.* 1947 *169* 247–258.

Bremer J. and D.M Greenberg, Biosynthesis of choline *in vitro*. *Biochim. Biophys. Acta.* 1960. *37* 173–175

Bremer J and D.M. Greenberg, Methyltransfering enzyme system of microsomes in the biosynthesis of lecithin (phosphatidylcholine). *Biochim. Biophys. Acta.* 1961 *46*. 205–216.

Browning, E.T Fluorometric enzyme assay for choline and acetylcholine. *Anal. Biochem.* 1972. *46*. 624–638

Campbell, L.B., I. Hanin and D.J Jenden, Gas chromatographic evaluation of the effects of some muscarinic and antimuscarinic drugs on acetylcholine levels in rat brain. *Biochem. Pharmacol.* 1970 *19* 2053–2059

Chakrin, L.W and V.P Whittaker The subcellular distribution of (N Me ^{3}H)acetylcholine synthesized by brain *in vitro*. *Biochem. J* 1969 *113* 97–107

Collier B. and C. Lang, The metabolism of choline by a sympathetic ganglion. *Can. J Physiol. Pharmacol.* 1969 *47* 119–126.

Collier B. and F.C. MacIntosh, The source of choline for acetylcholine synthesis in a sympathetic ganglion. *Can. J Physiol. Pharmacol.* 1969 *47* 127–135

Collier B., The preferential release of newly synthesized transmitter by a sympathetic ganglion. *J Physiol.* 1969 *205* 341–352.

Collier B., P Poon and S. Salehmoghaddam, The formation of choline and of acetylcholine by brain *in vitro*. *J Neurochem.* 1972. *19* 51–60

Consolo, S., H. Ladinsky G. Peri and S. Garattini, Effect of central stimulants and depressants on mouse brain acetylcholine and choline levels. *Eur J Pharmacol.* 1972. *18* 251–255

Crossland, J and A.J Merrick, The effect of anaesthesia on the acetylcholine content of brain. *J Physiol.* 1954 *125* 56–66.

Dawson, R.M.C., The metabolism of animal phospholipids and their turnover in cell membranes. In *Essays in Biochemistry* Vol. 2. Academic Press, London 1966. pp. 69–115

De Robertis, E., L. Salganicoff, L. Zieher and G Rodriguez de Lores Arnaiz, Acetylcholine and cholinacetylase content of synaptic vesicles. *Science.* 1963. *140* 300–301

Diamond, I. and E.P Kennedy Carrier mediated transport of choline into synaptic nerve endings. *J Biol. Chem.* 1969 *244* 3258–3263.

Diamond, I., Choline metabolism in brain. *Arch. Neurol.* 1971 *24* 333–339

Diamond, I. and D Milfay Uptake of (^{3}H-Methyl)choline by microsomal, synaptosomal, mitochondrial and synaptic vesicle fractions of rat brain. *J Neurochem.* 1972. *19* 1899–1909

Dross, K. and H. Kewitz, Concentration and origin of choline in the rat brain. *Naunyn-Schmiedeberg's Arch. Pharmakol. Exp. Pathol.* 1972. *274* 91–106.

Eade, I., C. Hebb and S.P Mann, Free choline levels in the rat brain (short communication). *J Neurochem.* 1973. *20* 1499–1502.

Fonnum, F The compartmentation of choline acetyltransferase within the synaptosome. *Biochem. J* 1967 *103* 262–270.

Giarman, N.J and G Pepeu, Drug-induced changes in brain acetylcholine. *Brit. J Pharmacol.* 1962. *19* 226–234

Haines, D S.M., The effect of choline deficiency and choline refeeding upon the metabolism of plasma and liver lipids. *Can. J Biochem.* 1966. *44* 45–57

Hanin, I., R. Massarelli and E. Costa, Environmental and technical preconditions influencing choline and acetylcholine concentration in rat brain. In *Drugs and cholinergic mechanisms in the CNS* Eds E. Heilbronn and A. Winter Försvarets forskningsanstalt, Almqvist and Wiksell, Stockholm. 1970. pp. 33–57

Hanin, I., R. Massarelli and E. Costa, An approach to the *in vivo* study of acetylcholine turnover in rat salivary glands by radio gas chromatography *J Pharmacol. Exp. Ther* 1972. *181* 10–18.

Hebb, C.O and B.N Smallman, Intracellular distribution of choline acetylase. *J Physiol.* 1956. *134* 385–392.

Hebb, C.O and V P Whittaker Intracellular distribution of acetylcholine and choline acetylase. *J Physiol.* 1958. *142* 187–196.

Hebb, C O The metabolism of (Me ^{14}C)choline in the brain of the rat *in vivo*. Personal communication to G.B. Ansell and S. Spanner *Biochem. J* 1968 *110.* 201–206.

Heilbronn, E. and B. Carlsson, Qualitative separation of choline esters by means of high voltage paper electrophoresis. *J Chromatog* 1960. *4* 257–259

Hodgkin, A.L. and K. Martin, Choline uptake by giant axons of Loligo. *J Physiol.* 1965 *179* 26–27
Holmstedt, B., G. Lundgren and A. Sundwall, Tremorine and atropine effects on brain acetylcholine. *Life Sci.* 1963 *10* 731–736.
Howes, J.F. L.S. Harris and W.L. Dewey The effect of morphine, nalorphine, naloxone, pentazoine, cyclazocine and oxotremorine on the synthesis and release of acetylcholine by mouse cerebral cortex slices *in vitro*, *Arch. Int. Pharmacodyn. Therap.* 1970. *184* 267–276.
Jenden, D Personal communication. 1972.
Kadota, K. and T Kanaseki, Isolation of a synaptic vesicle fraction from guinea pig brain with the use of DEAE Sephadex column chromatography and some of its properties. *J Biol. Chem.* 1969 *65* 839–842.
Kaita, A.A. and A.M. Goldberg, Control of acetylcholine synthesis – the inhibition of choline acetyltransferase by acetylcholine. *J Biochem.* 1969 *16* 1185–1191
Karlén, B., G Lundgren, I. Nordström and B. Holmstedt, Ion pair extraction in combination with gas phase analysis of acetylcholine. Collegium Int. Neuro-Psychopharmacologicum 1972. To be published.
Katz, H.S., S. Salehmoghaddam and B. Collier The accumulation of radioactive acetylcholine by a sympathetic ganglion and by brain failure to label endogenous stores. *J Neurochem.* 1973 *20* 569–579
Kennedy E.P and S.B. Weiss, The function of cytidine coenzymes in the biosynthesis of phospholipids. *J Biol. Chem.* 1956. *222* 193–214.
Kopin, I.J G.R. Breese, K.R. Krauss and V K. Weise, Selective release of newly synthesized norepinephrine from the cat spleen during sympathetic nerve stimulation. *J Pharmacol. Exp. Ther* 1968. *161* 271–278.
Korey S.R., B. de Braganza and D Nachmansohn, Choline acetylase. V esterifications and transacetylations. *J Biol. Chem.* 1951. *189* 705–715
Korkes, S., A. Campillo, S.R. del Korey J.R. Stern, D Nachmansohn and S. Ochoa, Coupling of acetyl donor systems with choline acetylase. *J Biol. Chem.* 1952. *198* 215–220.
Kuhar M.J., V.H. Sethy R.H. Roth and G.K. Aghajanian, Choline selective accumulation by central cholinergic neurons. *J Neurochem.* 1973 *20* 581–593
Kurokawa, M., Y Machiyama and M. Kato, Distribution of acetylcholine in the brain during various states of activity *J Neurochem.* 1963. *10* 341–348.
MacIntosh, F C., Synthesis and storage of acetylcholine in nervous tissue. *Can. J Biochem. Physiol.* 1963 *41* 2555–2571
Marchbanks, R.M., Exchangeability of radioactive acetylcholine with the bound acetylcholine of synaptosomes and synaptic vesicles. *Biochem. J* 1968 a. *106*, 87–95
Marchbanks, R.M., The uptake of (^{14}C)choline into synaptosomes *in vitro*. *Biochem. J* 1968 b. *110* 533–541
Marchbanks, R.M., The conversion of ^{14}C-choline to ^{14}C-acetylcholine in synaptosomes *in vitro*. *Biochem. Pharmacol.* 1969 *18* 1763–1766.
Marshall, E.F T Chojnacki and G.B. Ansell, The methylation of (^{32}P) phosphatidylmonomethylaminoethanol by S-adenosyl-L-(Me-^{14}C)methionine in liver preparations. *Biochem. J* 1965 *95* 30–31
Martin, K., Concentrative accumulation of choline by human erythrocytes. *J gen. Physiol.* 1968. *51* 497–516.
McCaman, R.E., Intermediary metabolism of phospholipids in brain tissue. *J Biol. Chem.* 1962. *237* 672–676.

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 398

HISTOCHEMICAL AND ELECTRON MICROSCOPIC OBSERVATIONS ON THE DEVELOPMENT, NEURAL CONTROL AND FUNCTION OF THE PANETH CELLS OF THE MOUSE

FROM THE DEPARTMENT OF ANATOMY UNIVERSITY OF HELSINKI,
SILTAVUORENPENGER 20, HELSINKI 17 FINLAND

HELSINKI 1973

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 398

HISTOCHEMICAL AND ELECTRON MICROSCOPIC OBSERVATIONS ON THE DEVELOPMENT NEURAL CONTROL AND FUNCTION OF THE PANETH CELLS OF THE MOUSE

BY
ANTTI AHONEN

FROM THE DEPARTMENT OF ANATOMY UNIVERSITY OF HELSINKI
SILTAVUORENPENGER 20 HELSINKI 17 FINLAND

HELSINKI 1973

Helsinki 1973 Erikoispaino Oy

PREFACE

I will express my deep gratitude to Professor Olavi Eränkö M.D Ph.D and chief of the Department for his valuable criticism and patient guidance during this work.

I am particularly indebted to Dr Antti Penttilä M.D., M.Sc. who proposed me the subject in 1968 and guided me during the first years of this work. Warm thanks to Professor Arto Palkama M.D Professor Martti Kormano M.D Docent Leena Rechardt, M.D and Dr Juhani Juntunen, M.L. for many advices and help

Many thanks to Docent Matti Lempinen M.D and Professor Kai Krohn M.D for reading the manuscript.

Special thanks to Miss Raisa Männikkö Mrs Liisa Kangasvuo Mrs Tuula Stjernvall and Mrs Eija Vuorio for their skilfull technical assistance. Thanks to Miss Helmi Palomäki, Librarian of the Department, for help in obtaining the literature and Mrs S Ruusamo M.Sc. for correcting my English I will further thank all the members of the staff for the stimulating spirit in the Department

My best thanks are due to my wife Eeva and our children Mia and Mikko for the patient support during this work.

Espoo 11 8 1973

Antti Ahonen

CONTENTS

INTRODUCTION 7

REVIEW OF THE LITERATURE
— Distribution of the Paneth cells 7
— Morphology of the Paneth cells 7
— Histochemistry of the Paneth cells 8
— Development of the Paneth cells 8
— Function of the Paneth cells 9
— Effect of drugs on the Paneth cells 9

PURPOSE OF THE PRESENT STUDY 10

GENERAL MATERIAL AND METHODS
— Material 10
— Histological methods 10
— Fluorescence histochemical methods 10
— Electron microscopic methods 11
— Histochemical reactions 11

PART ONE

I DEVELOPMENT OF THE PANETH CELLS OF THE MOUSE
— Introduction 12
— Material and methods 12
— Results 12
— Discussion 13

II. PANETH CELLS OF THE NORMAL MOUSE COLON
— Introduction 14
— Material and methods 14
— Results 15
— Discussion 16

PART TWO

III. EFFECT OF VAGOTOMY ON THE PANETH CELLS OF THE MOUSE
— Introduction 16
— Material and methods 17
— Results 18
— Discussion 18

IV EFFECT OF CHEMICAL SYMPATHECTOMY WITH 6-HYDROXYDOPAMINE ON THE PANETH CELLS OF THE MOUSE
— Introduction 20
— Material and methods 20
— Results 21
— Discussion 22

V EFFECT OF DECARBOXYLASE INHIBITION ON THE UPTAKE OF L-DOPA BY THE PANETH CELLS OF THE MOUSE AND CRYPT BASEMENT MEMBRANE
— Introduction 24
— Material and methods 24
— Results 24
— Discussion 25

PART THREE

VI ORTHO-PHTHALALDEHYDE INDUCED FLUORESCENCE IN THE PANETH CELLS OF THE MOUSE
— Introduction 26
— Material and methods 27
— Results 27
— Discussion 28

VII GENERAL DISCUSSION
— Uptake mechanism and secretion 29
— Protein synthesis and amino acid secretion 30
— Common origin of enterochromaffin -like cells and Paneth cells 30
— Colitis ulcerosa and Paneth cells 31
— Crypt basement membrane 32
— Crypt morphology and Paneth cells 32

MAIN NEW RESULTS AND CONCLUSIONS 33

FIGURES 35

REFERENCES 65

INTRODUCTION

The pyramidal cells at the bottom of the small intestinal crypts have been first described by Schwalbe (1872) and a rather profound reinvestigation at the light microscope level was made later by Paneth (1888) First electron microscope investigation of these cells was made by Hally (1958)

It has been estimated that average human intestine contains about 200 000 000 Paneth cells (Toner 1968) The number of Paneth cells increases in some pathological conditions (Lauren 1961 Toner 1968 Otto and Fett 1972)

Although Paneth cells may secrete enormous quantities of enzymes into the intestinal fluid the precise nature of the secretion is still unknown. Nor it is known if the secretion of the Paneth cells is under neural or hormonal control and at what age the secretion begins It is not known what role the Paneth cells have in the pathological conditions where they appear in the human stomach or colon (Lauren 1961 Toner 1968 Otto and Fett 1972)

REVIEW OF THE LITERATURE

Distribution of the Paneth cells

Paneth cells are present in about 50 % duodenal and 75 % of ileal and jejunal crypts (Toner 1968) and they are always situated at the bottom of the crypts and granule containing Paneth cells have never been seen to be higher up in the villi (Deschner 1967 Cheng et al 1969 Trougton and Trier 1969)

Paneth cells are present in man the mouse rat, guinea pig (Taylor and Flaa 1964) and ant bear (de Castro et al. 1952) rabbit (Pitha 1968) and some other species (Schaaf and Wenzel 1970) but no Paneth cells have been found in the cat, dog and birds (Taylor and Flaa 1964)

Morphology of the Paneth cells

Paneth cells are pyramidal cells which have microvilli in their apical parts projecting into crypt lumen Nucleus of the cells is often irregular and nucleolus is well developed (Hally 1958 Kurosumi 1961 Behnke and Moe 1964)

V EFFECT OF DECARBOXYLASE INHIBITION ON THE UPTAKE OF L-DOPA BY THE PANETH CELLS OF THE MOUSE AND CRYPT BASEMENT MEMBRANE
— Introduction 24
— Material and methods 24
— Results 24
— Discussion 25

PART THREE

VI. ORTHO-PHTHALALDEHYDE INDUCED FLUORESCENCE IN THE PANETH CELLS OF THE MOUSE
— Introduction 26
— Material and methods 27
— Results 27
— Discussion 28

VII GENERAL DISCUSSION
— Uptake mechanism and secretion 29
— Protein synthesis and amino acid secretion 30
— Common origin of enterochromaffin -like cells and Paneth cells 30
— Colitis ulcerosa and Paneth cells 31
— Crypt basement membrane 32
— Crypt morphology and Paneth cells 32

MAIN NEW RESULTS AND CONCLUSIONS 33

FIGURES 35

REFERENCES 65

(Deschner 1967 Cheng et al. 1969 Troughton and Trier 1969 Browning and Trier 1969) However the cells die and a new cell replaces the old the generation time being about 3 weeks (Cheng et al. 1969) Paneth cells possibly originate from less differentiated intermediate cells (Cheng et al. 1969 Sperschneider et al. 1972)

Function of the Paneth cells

It has been assumed that the function of the Paneth cells is to secrete digestive enzymes into the intestinal lumen (Hally 1958) especially peptidases (Behnke and Moe 1964 Kurosumi 1961) to secrete heavy metals (Halbhuber et al. 1970) to phagocytose bacteria (Erlandsen and Chase 1972 a, b) and to secrete amino acids (Gent and Creamer 1972) These or similar cells are found in the stomach and the colon under some pathological conditions (Lauren 1961 Toner 1968 Otto and Fett 1972) Lipids and proteins in the intestinal lumen have been shown to stimulate the secretion of the Paneth cells (Hally 1958) Milk diet increases the number of Paneth cells significantly (Recknagel et al. 1972)

Effect of drugs on the Paneth cells

Pilocarpine stimulates the secretion of the Paneth cells (Stanley and Trier 1965 Trier et al. 1967 Ahonen 1970) whereas atropine inhibits it (Stanley and Trier 1965 Trier et al. 1967) The Paneth cells of the mouse are able to take up catecholamines or their precursors (Penttilä and Ahonen 1969 Ahonen and Penttilä 1969 1970 1971) This uptake can be intensified by monoamine oxidase inhibition and prevented by imipramine and cocaine (Ahonen and Penttilä 1970 1971) Paneth cells are also able to take up labelled leucine (Stanley and Trier 1965 Trier et al. 1967) glycine methionine cysteine and tryptophan (Merzel 1967) glucose and arginine (Halbhuber et al. 1972) and heavy metals (Halbhuber et al. 1970) A total protein synthesis inhibition for two weeks decreases the number of Paneth cells and the number of granules per cell but is not capable of abolishing the typical secretory granules from the cells (Eisenhuth and Geyer 1966)

PURPOSE OF THE PRESENT STUDY

Present study was undertaken to resolve

- — whether the Paneth cells were under neural sympathetic and parasympathetic control
- — at what time does the normal Paneth cell secretion begin
- — whether there are cells related to small intestinal Paneth cells in the mouse colon
- — whether there is decarboxylase activity in the Paneth cells of the mouse
- — which cells exhibit ortho-phthalaldehyde induced fluorescence in the intestinum of the mouse.

GENERAL MATERIAL AND METHODS

The material consisted of 317 albino mice of both sexes of an randomly bred strain used in the Department of Anatomy The age of the experimental animals was from 5 days to six weeks The animals were fasted 24 hours before killing but they were allowed to drink tap water ad libidum.

Histological methods

For histological methods tissue samples were fixed with 3.5 % formaldehyde in 0 1 M phosphate buffer pH 7.2 dehydrated embedded in paraffin wax and the sections were stained with Best s carmine (Lauren et al. 1967) and Thioflavine T (Burns and Whitehead 1966) For other histological staining reactions the standard histological methods were used. Thioflavine T stained sections were viewed with fluorescence microscope (see below) All the other histologial sections were viewed with the usual light microscope.

Fluorescence histochemical methods

For fluorescence histochemical methods the mice were fasted one day before the experiment and they were given nialamide (Niamid® Pfizer) 500 mg/kg body weight intraperitoneally and about one hour later dopamine

chloride (Orion) 160 mg/kg intraperitoneally The drugs were dissolved in physiologic saline solution. The animals were killed at different intervals after dopamine injection In each group there were 3—4 mice Specimens were taken from the duodenum jejunum, ileum and colon frozen in isopentane precooled with liquid nitrogen freeze-dried in vacuo at —40°C in the presence of phosphorus pentoxide for two days heated to room temperature in vacuo for 4 hours and thereafter kept in vapour derived from para formaldehyde at 80°C for one hour The relative humidity of the formal dehyde vapour was equilibrated to 60 % The specimens were embedded in Epon, sectioned with LKB Pyramitome mounted in entellan and viewed with Leitz Ortholux fluorescence microscope provided with BG 3 and BG 12 and TAL 405 exitation and 470 nm barrier filters (for ref see Eränkö 1967)

Electron microscopic methods

For electron microscopy 2—3 small tissue pieces were taken from different parts of the colon and fixed with ice cold 3 5 % glutaraldehyde in 0 1 M phosphate buffer pH 7.2 for 2 hours and postfixed with 1 % osmium tetroxide in 0 1 M phosphate buffer after which they were rinsed with phosphate buffer embedded in Epon Araldite sectioned with LKB ultrotome, poststained with uranyl acetate and lead citrate and viewed with AEI 801 electron microscope.

Histochemical reactions

To control vagotomy histochemical acetylcholinesterase and non-specific cholinesterase methods (Koelle 1951) were used. To demonstrate acetyl cholinesterase activity acetylthiocholine iodine (Fluka) was used as substrate and iso-OMPA (10^{-5} M) as inhibitor and to demonstrate non-specific cholinesterase butyrylthiocholine iodine was used as substrate and 1,5-bis-(4-allyldimethyl-ammoniumphenyl)pentan-3-one-di iodine (10^{-5} M) as inhibitors respectively

The term *Secretion* means in the present investigation the disappearance of the secretory granule from the apical cytoplasm of the Paneth cell into the crypt lumen

PURPOSE OF THE PRESENT STUDY

Present study was undertaken to resolve

- whether the Paneth cells were under neural sympathetic and parasympathetic control
- at what time does the normal Paneth cell secretion begin
- whether there are cells related to small intestinal Paneth cells in the mouse colon
- whether there is decarboxylase activity in the Paneth cells of the mouse
- which cells exhibit ortho-phthalaldehyde induced fluorscence in the intestinum of the mouse

GENERAL MATERIAL AND METHODS

The material consisted of 317 albino mice of both sexes of an randomly bred strain used in the Department of Anatomy The age of the experimental animals was from 5 days to six weeks. The animals were fasted 24 hours before killing but they were allowed to drink tap water ad libidum

Histological methods

For histological methods tissue samples were fixed with 3.5 % formaldehyde in 0 1 M phosphate buffer pH 7.2 dehydrated embedded in paraffin wax and the sections were stained with Best s carmine (Lauren et al. 1967) and Thioflavine T (Burns and Whitehead 1966) For other histological staining reactions the standard histological methods were used. Thioflavine T stained sections were viewed with fluorescence microscope (see below) All the other histologial sections were viewed with the usual light microscope

Fluorescence histochemical methods

For fluorescence histochemical methods the mice were fasted one day before the experiment and they were given nialamide (Niamid[R] Pfizer) 500 mg/kg body weight intrapentoneally and about one hour later dopamine

cells visible in the small intestine in contrast to the colon where such cells were numerous at that time (Fig. 1 and 2) Some eosinophil and lymphoid cells were also visible in the crypts at the age of 2—3 weeks (Fig 5)

By electron microscopy the Paneth cells were demonstrable in the small intestine of the mouse after the first week of development at same time when the intestinal crypts began to develop Intestinal villi had already developed some time earlier At the age of 1 3 weeks the Paneth cells reached their normal adult morphology showing the characteristic electron-dense secretory granules well-developed Golgi complexes and abundant rough endoplasmic reticulum (Fig. 7 and 8) The first Paneth cells were often long and narrow and consisted of many large mitochondria of different shapes (Fig 8 and 9) There were regularly also some small electron dense bodies in the Golgi region (Fig. 7 and 9) The small early Paneth cell granules had a vide regular halo which disappeared when the granules had grown (Fig 7 and 9) The diameter of the Paneth cell granules was 1—3 microns and the older Paneth cells had larger granules (Fig 7 and 9)

During the first 3 weeks of development the Paneth cells of the mouse did not take up dopamine but started to do so at the age of 3 weeks (Fig. 6) This dopamine uptake reached its adult level at the age of 4 weeks.

Discussion

The results of the present work are in agreement with the earlier obser vations that Paneth cells of the mouse appear at about the age of 1 week after the birth (Merzel 1967 Sperschneider et al. 1972) In the rat these cells appear about 1 week later (Toner 1968) According to the present work the appearance of the Paneth cells into the small intestinum took place about at the same time as the beginning of the crypt development, which may be considered as a support of the hypothesis proposed by Creamer (1967) that Paneth cells are responsible for the normal morphology of the small intestinum.

It has been proposed that Paneth cells originate from the slender inter mediate cells of the crypt which take up labelled thymidine within some hours in contrast to Paneth cells which take up label only some days after the single injection (Cheng et al. 1969 Trougton and Trier 1969 Recknagel et al. 1972) These intermediate cells may possibly also become transformed into the goblet cells (Merzel and Leblond 1969) However the precise origin of the Paneth cells remains obscure in the present work.

Dopamine taken up by the Paneth cells of the mouse seems to be rather tightly bound to the granules and disappears from the cells into the intestinal lumen when the whole granule is secreted (Ahonen and Penttilä 1971) It is possible that dopamine uptake and secretion of the granule marks the process of granule synthesis and secretion, which thus can be visualized by formaldehyde induced fluorescence due to dopamine. This accumulation and secretion of dopamine is similar in function of time to that received with labelled leucine (Trier at al. 1969) and glucose but different from that received with labelled arginine (Halbhuber et al. 1972) Thus the beginning of the dopamine uptake by the Paneth cells of the mouse at the age of 3 weeks can possibly be interpreted as the beginning of the normal function of the Paneth cells The dopamine uptake reached its normal adult level at the age of 4 weeks which may reflect the normal adult secretion capacity of the Paneth cells Simultaneously with the development of the uptake mechanism also adrenergic nerve fibers appeared into the intestinum (Mirkin 1972) as well as the first lymphocytes at the age of 3 weeks (Bäck 1970 1972)

II PANETH CELLS OF THE NORMAL COLON OF THE MOUSE

Introduction

Paneth cells are able to phagocytose bacteria (Erlandsen and Chase 1972 a b) and their granules contain lysosomal enzymes (Riecken and Pearse 1966) and lysozyme (Deckx et al. 1967 Ghoos and Vantrappen 1971) Under zinc free diet bacterial invasion into the Paneth cells takes place (Otto and Weitz 1972)

There is some controversy about the presence of Paneth cells in a normal colon Instead they appear rather regularly into the human colon during ulcerative colitis (Otto and Fett 1972) The purpose of the present work is to study the presence of the Paneth cells in the normal colon of the mouse

Material and methods

The material consisted of 26 adult albino mice of both sexes For histological electron microscopical and fluorescence histochemical methods the samples were taken from different parts of the colon and treated according to methods described in details in general material and methods

Results

Characteristics of the basally located epithelial cells in the colonic crypts of the mouse were poorly visible with the usual staining methods (Fig 10 and 11) Neither was it possible to differentiate the basal epithelial cells from goblet cells accurately by Best s carmine or iron hematoxylin techniques Best identification of the basally located epithelial cells which were called colonic Paneth cells in the present work was obtained by staining with Thioflavine T and fluorescence microscopy after formaldehyde fixation. This method stained also the goblet cells (Fig. 12 13 14 and 15) and it was therefore sometimes difficult to identify the colonic Paneth cells The granules of the colonic Paneth cells stained strongly with periodic acid-Schiff technique.

In the freeze-dried Epon sections stained with toluidine blue dark stained granular cells appeared higher up in the crypts (Fig 16) These dark granulated cells were distinctly granular and seemed to be slightly different from the goblet cells

In the colon of the adult non-treated mouse there was no formaldehyde-induced fluorescence (FIF) except in nerve fibers and enterochromaffin and mast cells. After nialamide and dopamine injection, strongly fluorescent, granular cells appeared at the bottom of the colonic crypts of the mouse. (Fig. 17 18 19 and 20) The size of the fluorescent granules varied greatly (Fig. 19 and 20) The fluorescence disappeared from the colonic epithelial cells within 3—4 hours and this loss was probably due to secretion of fluorescent granules into the cryptal lumen. The coliformic bacteria of the large bowel seemed to be rather near the basal epithelial cells but they never invaded these cells (Fig 21)

By electron microscopy the basal epithelial cells in the colon of the mouse were observed to be distinctly different from the small intestinal Paneth cells The granules were pale and there were more granules per cell than in the small intestine There were also some smaller and darker granules in these cells (Fig. 21) The basal epithelial cells had a great nucleous and a well developed Golgi apparatus and a darkly stained endoplastic reticulum. Single ribosomes could not be seen in the endoplasmic reticulum so that it is difficult to say whether the endoplasmic reticulum had a smooth or rough surface but the universal strong staining may indicate the presence of the rough endoplasmic reticulum like in the small intestinal Paneth cells (Fig. 21)

Discussion

The ability of the Paneth cells in the small intestine of the mouse to take up L-dopa and dopamine was first described by Penttilä and Ahonen (1969) This accumulation of dopamine can be intensified by monoamine oxidase inhibition and prevented by cocaine and imipramine (Ahonen and Penttilä 1969 1970 1971) According to the present study both the Paneth cells of the small intestine and the epithelial cells at the bottom of the colonic crypts normally have no histochemically demonstrable catecholamines but take up dopamine becoming distinctly visible by FIF The ability of the cells to take up monoamines and their precursors has been proposed possibly to reflect the neural crest origin of the cells (Pearse et al. 1969) Epithelial cells at the bottom of the colonic crypts were morphologically slightly different from the small intestinal Paneth cells but had the same capacity to take up dopamine. Therefore it is proposed that Paneth cells of the small intestine and the basal epithelial cells in the colonic crypts are embryologically related and, accordingly the basal epithelial cells in the colonic crypts can be called colonic Paneth cells Cells resembling the small intestinal Paneth cells appear into the human colon during ulcerative colitis (Otto and Fett 1972) According to the present study the number of colonic Paneth cells per surface area is greatest at the taenial region (Fig. 13) Ulcerations in the ulcerative colitis are also often situated at the taenial region (Boyd 1961)

PART TWO

III EFFECT OF VAGOTOMY ON THE PANETH CELLS OF THE MOUSE

Introduction

It has been assumed that Paneth cells secrete digestive enzymes and that fat and protein in the intestinum stimulates the secretion of the Paneth cells (Hally 1958) Milk diet doubles the number of the Paneth cells (Recknagel et al 1972) Paneth cells have a high basal secretion rate impossible to be disturbed significantly by normal diet (Trier et al. 1967) They can phagocytose bacteria (Erlandsen and Chase 1972 a b) and they can take up catecholamines (Penttilä and Ahonen 1969) This uptake can be intensified with nialamide or pargyline and it is prevented by imipramine and cocaine (Ahonen and Penttilä 1970 1971) The Paneth cells can also take up cysteine and tryptophan (Merzel 1967) Pilocarpine stimulates the

secretion of the Paneth cells (Stanley and Trier 1965 Trier et al 1967 Ahonen 1970) and atropine inhibits it (Trier et al. 1967)

In the present study the effect of bilateral vagotomy and dopamine on the Paneth cells of the mouse was studied, since vagotomy can be expected to influence the Paneth cells but no previous study is available dealing with this subject.

Material and methods

Total 90 adult albino mice of both sexes were used in the present experiment. Vagotomy was made by dissecting and removing both the anterior and the posterior trunks of the vagus nerves at the oesophagus a little above the cardia under aether anesthesia. The animals were killed 16—24 hours later by cervical dislocation and the samples were taken from the middle part of duodenum and the mid jejunum

For fluorescence histochemical methods the mice were given intraperit oneally nialamide (Niamid[R] Pfizer) 500 mg/kg and 1 hour later dopamine chloride (Orion) 160 mg/kg intraperitoneally The animals were killed 15 30 and 60 min., 2 3 4 5 and 6 hours after dopamine injection in both vagotomized as well as the control groups. In each group there were 3—4 mice The specimens were treated for histological and fluorescence histochemical methods according to the standard methods described in details in general material and methods

The number of the secretory granules per cell was counted in a total of 600 duodenal and jejunal Paneth cells The calculation in each group was made in 4 different animals and 6 adjacent 7 μ thick sections No thickness correction was made, because the intention was to obtain relative figures rather than the exact number of granules per cell. The reliability of the calculation method was tested by making two different controls at an interval of one month, and between these controls there was no significant difference. Student t test was used in statistical analyses

Vagotomy was controlled by histochemical acetylcholinesterase and non-specific cholinesterase methods (Koelle 1951) After vagotomy the acetyl cholinesterase positive nerve fibers disappeared from the muscle, from the submucous layer as well as from the crypt basement membrane where they were in close relationship with the crypt basement membrane in the control group (Fig 26 27 and 28) In the control group there was a positive non-specific cholinesterase reaction in the nerve fibers near the crypt basement membrane (Fig 26) and also in the Paneth cells and the other epithelial cells. The non-specific cholinesterase reaction became stronger in the nerve cells in the submucosa after vagotomy

Results

According to the present work the secretory granules of the Paneth cells of the mouse showed a weak masked metachromasia (Fig 22) Normally the Paneth cell granules did not have formaldehyde-induced fluorescence (FIF) but they were well visible with Thioflavine T fluorescence instead (Fig 24) After nialamide (500 mg/kg) and dopamine (160 mg/kg) the green fluorescence appeared in the apical secretory granules within 10 minutes (Fig 23) The number of fluorescent granules increased thereafter (Fig 25) reaching its maximum about 1 hour later The secretion of the fluorescent granules into the intestinal lumen began about 1 hour after the dopamine injection and all the fluorescent granules left the Paneth cells from 3 to 4 hours after the dopamine (Table I) After vagotomy the fluorescent granules left the Paneth cell about 1 hour later (Table I)

The effect of vagotomy on the Paneth cells of the mouse was also studied by calculating the average number of granules per cell before and one day after vagotomy and it was observed that vagotomy increased the average number of granules per cell from 12 1 ± 3.2 to 13.8 ± 3 3 in the duodenum and from 8 0 ± 1 8 to 12.8 ± 3 0 in the jejunum (Fig 30 and 31) Both differences were significant ($p < 0\ 001$) After vagotomy the Paneth cell granules were also a little bigger with a large halo than in the control group (Fig 29) Dopamine decreased the granule content both in the duodenum and in the jejunum significantly ($p < 0\ 001$) The decrease was from 12 1 ± 3.2 to 9 1 ± 2 6 in the duodenum and from 8 0 ± 1.8 to 6 6 ± 1 6 in the jejunum (Fig 30 and 31)

The average number of granules of the duodenal Paneth cells was significantly ($p < 0\ 001$) greater than the number of granules in the jejunal cells (Fig 30) The duodenal Paneth cell granules had a slightly higher turnover time than the jejunal cell granules (Table I) A conclusion may be drawn from the calculations of granules that dopamine by decreasing the number of granules per cell significantly stimulates the secretion of the Paneth cells and had also influence in the results in Table I This stimulating effect was however similar in the experiment as well as the control groups and thus the results are comparable with each other In reality the basal secretion rate in the Paneth cells of the mouse was therefore, a little slower than the results in Table I

Discussion

Pilocarpine stimulates and atropine inhibits secretion of the Paneth cells when studied by labelled leucine (Stanley and Trier 1965 Trier et al. 1967) The present results with vagotomy further supports the idea that secretion

stimulus is mediated thorough parasympathetic, cholinergic vagus nerves The present results that dopamine stimulates the secretion may indicate that sympathetic stimulation perhaps exerts a regulatory effect on the secretion of the Paneth cells.

The turnover time of the Paneth cell granules reported in this paper with dopamine is very similar to those obtained with glucose and leucine (Halbhuber et al. 1972 Trier et al 1967) but differs distinctly from that obtained with arginine (Halbhuber et al. 1972) arginine appears in the Paneth cells much later after the injection and first becomes visible in the paranuclear region much below the Golgi apparatus It therefore seems likely that dopamine, glucose and leucine may reflect active uptake and synthesis in the Paneth cells whereas arginine is possibly storaged as such or bound to some other substance outside the granules and is possibly used for the synthesis of the granules later

The close relation of the cholinergic nerves with the crypt basement membrane is similar to that observed by Pack et al (1967) and is similar to that observed in the gastric crypts (Lack et al. 1968) Atropine and vagotomy inhibits secretion of the hydrochloric acid by the parietal cells (Hirschowitz et al. 1972 Emås 1973) According to the present results vagotomy has similar effects on the Paneth cells as on the gastric parietal cells.

TABLE No I

Effect of vagotomy on the secretion of the Paneth cells of the mouse

Dopamine fluorescence was estimated at different intervals after nialamide (500 mg/kg) and dopamine (160 mg/kg) injection intraperitoneally in the Paneth cell granules of the mouse.

Expalenations — = no fluorescence, + = trace of fluorescence, ++ = weak fluorescence +++ = moderate fluorescence, and ++++ = strong fluorescence.

Time after dopamine	15 min	30 min	1 h	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
VAGOTOMY								
duodenum	+	++	+++	++++	+++	++	+	—
jejunum	+	++	+++	++++	+++	+++	+	—
CONTROL								
duodenum	++	+++	++++	++	+	—	—	—
jejunum	+	+++	++++	+++	++	+	—	—

IV EFFECT OF CHEMICAL SYMPATHECTOMY WITH 6-HYDROXY DOPAMINE ON THE PANETH CELLS OF THE MOUSE

Introduction

Paneth cells are more numerous in the jejunum and the ileum than in the duodenum (Toner 1968) whereas duodenal Paneth cells have more granules (Chapter III) It has been shown by electron microscopy that Paneth cells secrete their granules into the intestinal lumen where they dissolve rapidly (Hally 1958 Kurosumi 1961 Behnke and Moe 1964)

Pilocarpine (Stanley and Trier 1965 Trier et al 1967 Ahonen 1970) and dopamine (Chapter III) stimulate the secretion of the Paneth cells and vagotomy (Chapter III) and atropine inhibit it (Trier et al. 1967) The Paneth cell granules are able to take up catecholamines (Penttilä and Ahonen 1969 Ahonen and Penttilä 1969 1970 1971) cysteine and thryptophan (Merzel 1967) leucine (Trier et al. 1967) glucose and in some extent also arginine (Halhuber et al. 1972) Milk diet doubles the content of the Paneth cells of the mouse (Recknagel et al 1972) In the present study the effect of chemical sympathectomy on the Paneth cells was examined as there are no reports on the effects of sympathectomy on the Paneth cells available at present.

Material and methods

About 100 adult albino mice of both sexes were used. The animals were fasted one day before experiments and samples were taken from the mid-duodenum and midjejunum

Sympathectomy was made by injecting 50 mg of 6-hydroxydopamine chloride (Hoffman La Roche) in saline solution intraperitoneally 2—14 days before experiments Samples were taken from the mid-duodenum and mid-jejulum and stained with Thioflavine T and Best s carmine. Paneth cell granules were calculated as in Chapter III in 4 different animals and 6 adjacent sections and of total 600 Paneth cells in each group

For fluorescence histochemical methods the Paneth cell granules were labelled in the sympathectomized as well as the control groups by injecting intraperitoneally dopamine chloride (Orion) 160 mg/kg intraperitoneally 1 hour after nialamide (Niamid[R] Pfizer) 500 mg/kg. The animals were killed 5 10 15 30 45 and 60 minutes 2 3 4 5 and 6 hours after the dopamine injection. Specimens were taken and treated for formaldehyde-induced fluorescence according to standard methods.

Results

The formaldehyde-induced fluorescence (FIF) of the adrenergic nerve fibers of the intestinum totally disappeared after treatment with 6-hydr oxydopamine. After such sympathectomy the Paneth cell granules were greater than those in the control group and the whole cell was filled with granules (Fig 32)

The secretion speed of the Paneth cell granules was studied fluorescence histochemically with FIF after labelling the granules with dopamine, which is specifically taken up to these granules (Penttilä and Ahonen 1969 Ahonen and Penttilä 1971) After 6-hydroxydopamine injection the green fluor escence in the granules labelled with dopamine disappeared about 1 hour later than that in the control group (Table II)

Dopamine fluorescence accumulated in the Paneth cell granules at about the same rate in the 6-hydroxydopamine injected and in the control mice Five minutes after the dopamine injection the whole Paneth cell was visible as non-fluorescent against the other strongly fluorescent cells (Fig 33) Ten to fifteen minutes after the dopamine injection the fluorescent material began to accumulate in the Paneth cell granules in the Golgi region (Figs 34—37) and the nuclear membrane also became fluorescent and a large fluorescent particle probably nucleolus appeared inside the nucleus (Fig 34 and 35) At the same time, also weakly fluorescent granular cells appeared in the intestinal epithelium and the lamina propria (Fig 34 and 36) These cells were granular and stained with eosin. They were possibly eosinophil leukocytes which some time later also appeared higher up in the crypts and intestinal villi (Fig 38)

Two weeks after sympathectomy with 6-hydroxydopamine the crypt basement membrane began to deform and white material appeared beneath the basement membrane. At the same time the intestinal villi also began to deform In the most deformed crypts there were no typical Paneth cells left (Fig. 39)

The effect of chemical sympathectomy was also studied by calculating the average number of granules per cell in each group a total of 600 Paneth cells It showed that sympathectomy increased the number of granules per cell from $12\,1 \pm 3.2$ to $16.5 \pm 4\,1$ in the duodenum and from $8\,0 \pm 1.8$ to $17\,0 \pm 5.3$ in the jejunum. The differences were significant ($p < 0\,001$) Dopamine decreased the granule content signifficantly (Fig 40 and 41) As to the jejunal Paneth cells the effect of dopamine was greater after sympathectomy indicating that jejunal Paneth cells became sensitive againsti dopamine by 6-hydroxydopamine treatment Dopamine decreased the granule content from $17\,0 \pm 5\,3$ after sympathectomy to 10.8 ± 2.9 in the jejunum.

In the duodenum the decrease in the granule content caused by dopamine after sympathectomy was from 16.5 ± 4.1 to 13.7 ± 3.2 (Fig 40 and 41) All the differences were significant ($p < 0.001$) After sympathectomy the average number of granules per cell was about the same in the duodenal and in the jejunal Paneth cells ($0.1 > p > 0.05$) whereas the control group showed significantly higher ($p < 0.001$) number of granules per cell in the duodenum (Fig 40 and 41)

Discussion

The compound 6-hydroxydopamine produces a long lasting depletion of noradrenaline from peripheral sympathetically innerved tissues in various species The denervation takes place through degeneration and possibly disappearance of the peripheral adrenergic nerves (Uretsky and Iversen 1969 Hökfelt et al. 1972) During this sympathectomy acetylcholine or choline asetystransferase activities are not altered (Consolo et al. 1972)

According to the present study sympathectomy with 6-hydroxydopamine inhibited the secretion of the Paneth cells more than did vagotomy In the present study both duodenal and jejunal Paneth cells had the same capacity to form and secrete granules although the number of granules in the jejunal Paneth cells was normally smaller After sympathectomy the granules in the Paneth cells of the mouse were greater than in the mice of the control group after vagotomy which may mean that parasympathetic stimulus has greater effect on the granule synthesis as had been proposed also by Trier et al (1967) However the parasympathetic stimulus influences also the secretion process to some extent Sympathetic stimulus does apparently not have as great an effect on the synthesis but it is needed for the normal basal secretion.

After chemical sympathectomy the jejunal Paneth cells became sensitive towards dopamine This is in agreement with the observation that 6-hydroxydopamine produces supersensitivity to noradrenaline in perfused rat mesenterium (Finch and Leach 1970) and aorta of the rat (Shibata et al. 1972) and the sympathetic hyperinnervation produces hyposensitivity to noradrenaline (Kuzmicheva et al. 1969) Cyclic adenosine monophosphate has been shown to be a transmitter of the sympathetic stimulus in many organs (Takagi et al. 1971 Andersson 1972, Kaliner et al. 1972 Nakanishi and Takeda 1972 Smythies 1972) and 6-hydroxydopamine has been shown to produce an increased cyclic adenosine monophosphate response to noradrenaline (Palmer 1972) It can be therefore expected that cyclic adenosine monophosphate may serve as a modulator of adrenergic stimulation also in the Paneth cells of the mouse

The observation that dopamine is also bound to a great particle inside the nucleus which may be nucleolus may have some connections with the discovery that noradrenaline stimulates polyamine synthesis (Caldarera et al. 1970)

The presence of adrenergic nerve fibers in close contact with the crypt basement membrane has been shown earlier (Costa and Gabella 1967 Gabella and Costa 1968 Silva et al. 1968)

The presence of adrenergic-cholinergic interactions has been proposed by many authors (Åberg and Eränkö 1967 Rauanheimo and Eränkö 1968 Mori et al. 1968 Tacca et al. 1970 Elfin 1971 a, b c Kosterlitz and Leeds 1972 Consolo et al. 1972 Kanerva and Hervonen 1972) If such interactions exist in the nerves that regulate the secretion of the Paneth cells it seems reasonable that cholinergic stimulus may be modulated through adrenergic receptors because the specific adrenergic peripheral denervation with 6-hydroxydopamine the nature of which has been widely studied (Thoenen and Tranzer 1968 Uretsky and Iversen 1969 Finch and Leach 1970 Gosling and Dixon 1972 Hökfelt et al. 1972) had greater inhibitory action on the Paneth cells of the mouse than vagotomy inhibiting the secretion of the Paneth cells most.

TABLE No II

Effect of sympathectomy on the secretion of the Paneth cells of the mouse

Sympathectomy was made by injecting 50 mg/kg 6-hydroxydopamine intraperitoneally 2—14 days earlier Nialamide was injected intraperitoneally 500 mg/kg and dopamine 160 mg/kg one hour later Thereafter the animals were killed at different intervals after dopamine injection. The control group received only nialamide and dopamine. The intensity of fluorescence in the Paneth cell granules was estimated visually and each column is a mean value of 3—4 experiments.

Explanations: — = no fluorescence in the Paneth cell granules,
+ = trace of fluorescence, ++ = weak fluorescence,
+++ = moderate fluorescence and ++++ = strong fluorescence

Time after dopamine	10 min	30 min	45 min	60 min	2 hrs	3 hrs	4 hrs	5 hrs	6 hrs
SYMPATHECTOMY									
duodenum	+	++	++	+++	++++	++	+	—	—
jejunum	+	++	++	+++	++++	+++	++	+	—
CONTROL									
duodenum	+	++	+++	++++	++	+	—	—	—
jejunum	+	++	+++	++++	+++	++	+	—	—

V EFFECT OF DECARBOXYLASE INHIBITION ON THE L-DOPA UPTAKE BY THE PANETH CELLS OF THE MOUSE AND THE CRYPT BASEMENT MEMBRANE

Introduction

Paneth cells take up exogenous catecholamines in their special secretory granules and this uptake is intensified by monoamine oxidase inhibition and prevented by imipramine or cocaine (Penttilä and Ahonen 1969 Ahonen and Penttilä 1969 1970 1971) It has been proposed earlier that also Paneth cells belong to the APUD (Amine Precursor Uptake and Decarboxylation) cells (Ahonen and Penttilä 1971) In the present study the effect of decarboxylase inhibition on the L-dopa uptake was investigated by using the formaldehyde induced fluorescence (FIF) method for histochemical demonstration of catecholamines (Eränkö 1967) So far as I know no such study has been carried out earlier and it would be of interest to know if there is decarboxylase activity in the Paneth cells of the mouse because it is one of the properties of the APUD cells

Material and methods

The material consisted of 40 albino mice of both sexes of an unknown strain used in the Department of Anatomy

The following drugs were used nialamide (Niamid[R] Pfizer) 500 mg/kg intraperitoneally 1—2 hours before L-dopa or dopamine injection, the decarboxylase inhibitor Ro 4602 (Hoffman La Roche) 40 mg/kg intraperitoneally 30 minutes before L-dopa injection L-dopa (Orion) 160 mg/kg intravenously and dopamine chloride (Orion) 160 mg/kg intravenously Both L-dopa and dopamine were injected 5 15 30 60 minutes, 2 4 and 5 hours before killing the animals All the drugs were dissolved in physiologic saline. To dissolve L-dopa a small amount of 1 N HCl was used

Results

It was observed in the present work that leukocytes penetrate into the crypt lumen between two Paneth cells at the bottom of the crypt (Fig. 42) The Paneth cell cytoplasm was non-fluorescent immediately after dopamine injection in contrast to the other epithelial cells which were diffusely

fluorescent (Fig 43 and 45) At the same time there was also some large fluorescent particles visible in the nuclear region of the Paneth cells (Fig 43) Ten minutes after dopamine injection the fluorescent material began to accumulate in the apical secretory granules of the Paneth cells of the mouse (Fig. 44) The number of fluorescent secretory granules increased soon thereafter

The crypt basement membrane was also strongly fluorescent soon after dopamine injection and remained fluorescent for 1.5 hours (Fig 43 44 and 45) After chemical sympathectomy by 6-hydroxydopamine the crypt basement membrane was loosened from the epithelial cell lining and both the basement membrane and the cell membrane of the Paneth cells became fluorescent (Fig. 45)

After decarboxylase inhibition with Ro 4602 and L-dopa injection the cytoplasm of the Paneth cells and the crypt basement membrane remained negative whereas the other epithelial cells received their strong green diffuse fluorescence (Fig 46) No accumulation of the fluorescent material in the secretory granules took place (Fig 46) as compared to controls (Fig 44) The only green fluorescent cells after Ro 4602 and L-dopa injection were some mast cells and small eosinophil leukocytes which had some big strongly fluorescent granules visible higher up in the crypts and villi and sometimes also in the cryptal lumen (Fig 47)

Discussion

The Paneth cell granules are able to take up L-dopa and dopamine (Penttilä and Ahonen 1969) According to the present work it was possible to prevent the appearance of the L-dopa induced fluorescence in the Paneth cell granules by decarboxylase inhibition with Ro 4602 This suggests that some decarboxylase activity exists in the Paneth cells and that L-dopa cannot be taken up by these cells unless it is first decarboxylated to dopamine. The presumed decarboxylase acitivity in the Paneth cells would further support the earlier proposal that Paneth cells belong to the APUD (Amine Precursor Uptake and Decarboxylation) series of cells (Ahonen and Penttilä 1971)

The lack of fluorescence of the crypt basement membrane after Ro 4602 and L-dopa injection may mean the presence of decarboxylase activity in the crypt basement membrane. If there is however no decarboxylase activity in the basement membrane, the only explanation is that dopamine is bound

to the basement membrane substance inside the Paneth cells and transferred thereafter to the site of the basement membrane with a rather fast turnover time It has been shown that the epithelial basement membrane is universally secreted by adjacent epithelial cells (Pierce and Nakane 1972) which may favour the latter with the above possibilities but does not exclude the former

The enzymes needed for the production of melanine from L-dopa or L-tyrosine have been found in the eosinophil cells and the mast cells (Okun et al 1970) The accumulation of L-dopa into these cells after decarboxylase inhibition observed in the present work suggests that the production of melanin is favoured after decarboxylase inhibition with Ro 4602 in the small intestinum of the mouse. This may have some connections with the abnormal formation of melanin pigment which sometimes occurs in organs which normally have no pigments.

PART THREE

VI ORTHO PHTHALALDEHYDE INDUCED FLUORESCENCE IN THE PANETH CELLS OF THE MOUSE

Introduction

It is possible to demonstrate Paneth cells using formaldehyde-induced fluorescence because the cells take up dopamine and L-dopa into their secretory granules (Penttilä and Ahonen 1969 Ahonen and Penttilä 1969) and this uptake can be inteisified by monoamine oxidase inhibition and blocked by imipramine and cocaine (Ahonen and Penttilä 1970 1971)

The specificity of formaldehyde-induced fluorescence (FIF) first described by Eränkö in 1952 is well established (see Eränkö 1967) Ortho-phthalaldehyde induced fluorescence (OPTIF) has been shown to demonstrate histamine (Shore et al. 1959 Ehinger et al. 1968 Shelley et al. 1968 Shelley and Öhman 1968) histidine (Ehinger and Thunberg 1967) arginine, 5-hydroxyindoleamines secretin, glucagon and vasoactive intestinal peptide (Håkanson et al. 1971 Polak et al. 1971 Håkanson et al. 1972) OPTIF visualizes also mast cells (Ehinger et al. 1968 Shelley et al. 1968 Shelley and Öhman 1968) pancreatic islets (Takaya 1970) and sweat ducts (Öhman and Shelley 1969) In the present work the localization of OPTIF (Ehinger et al 1968 Enerbäck 1969 Takaya 1970) was studied in the intestinal cells by using a method in which epoxy resin sections were used to facilitate the localization of the reaction.

Material and methods

The material consisted of 21 adult albino mice of both sexes. The samples were taken from different parts of the intestinum and treated for formaldehyde-induced fluorescence according to standard methods (see general material and methods)

The Epon sections were viewed in the fluorescence microscope for catecholamines and provided with the same filters as earlier Thereafter the setions were moistened with a small amount of water to dissolve the FIF One drop of 1 % o-phthalaldehyde in xylene was put on the slide and the OPTIF was examined with the fluorescence microscope using the same filters If wanted, the same sections were stained later on with toluidine blue. The color of the FIF due to catecholamines was green and that of OPTIF yellowish Some sections were stained without being moistened with water and it was observed that no OPTIF developed.

All the experimental animals except those of the normal control group were given 500 mg/kg nialamide (Niamid[R] Pfizer) intraperitoneally 3—5 hours before and dopamine chloride (Orion) 160 mg/kg 15 and 30 minutes 1 2 3 and 4 hours before being killed.

Results

After abolishing the FIF (Fig 48 and 49) from a freeze-dried Epon section by a small amount of water it was possible to develoo OPTIF in the same section while observing it in the fluorescence microscope. Water abolished the FIF rapidly On the other hand, it was not possible to obtain OPTIF without moistening the section with a small amount of water Paneth cells were identified after dopamine injection by their green fluorescent granules and the enterochromaffin cells by their yellow FIF Without dopamine treatment the Paneth cells showed no FIF in their granules

The mast cells in the lamina propria were visible with both FIF and OPTIF (Fig 50) The secretory granules of the Paneth cells of the mouse were distinctly fluorescent with OPTIF also without dopamine pretreatment (Fig. 50 51 52 and 53) More granules per cell were visible with OPTIF as compared with FIF indicating that not all the Paneth cell granules take up dopamine after a single injection (Fig. 52)

Small granular cells appeared the cryptal lumen exhibiting a strong green FIF in their granules about 1 hour after dopamine injection. Later these granular cells were also visible higher up in the crypts and the lamina propria These granular cells which stained with eosin and exhibited also OPTIF were morphologically different form the mast cells (Fig 50 51) The

number of these OPTIF positive cells increased after dopamine injection

In the colonic Paneth cells the OPTIF was more diffuse and not so distinctly granular as in the small intestine The nerve fibers were non-fluorescent after the water and showed no OPTIF indicating that this was not due to catecholamines. The crypt basement membrane also exhibited a clear OPTIF (Fig 53)

Discussion

It was shown in the present study that OPTIF was demonstrable in the Paneth cell granules in the enterochromaffin cells and in the mast cells The Paneth cell granules are known to contain arginine and histidine (Taylor and Flaa 1964 Merzel 1967) It has been shown that both arginine and histidine have "protected linkages in the Paneth cell granules while no arginine and histidine can be found in the pancreatic acinar cells (Merzel 1967) This may mean that histidine and arginine are bound to peptides

Glucagon and secretin which also contain NH_2-terminal histidine also exhibit OPTIF whereas insulin the thyroid-stimulating hormone and thyrotropin releasing hormone which are devoid of histidine residue do not show OPTIF (Håkanson et al 1971 Håkanson et al 1972) The pancreatic islets show OPTIF and this is probably due to glucagon (Takaya 1970 Håkanson 1971) The OPTIF of the mast cells is probably not due to histamine (Ehinger et al. 1968 Shelley et al 1968 Shelley and Öhman 1968)

The OPTIF in the Paneth cell granules may be due to arginine, to histidine or to both of them The Paneth cells are the greatest cell population in the intestinum exhibiting OPTIF Thus they are probable site of the vasoactive intestinal peptide which also is OPTIF positive (Håkanson et al. 1972) It has been proposed earlier (Ahonen and Penttilä 1971) that Paneth cells belong to the polypeptide producing APUD (Amine Precursor Uptake and Decarboxylation) cell system of Pearse (1969) being able to take up catecholamines and their precursors Moreover it has been shown that there is decarboxylase activity in the Paneth cells (Chapter V) If the vasoactive intestinal peptide (Håkanson et al. 1972) is the polypeptide secreted by the Paneth cells it would be understandable that these cells belong to the APUD cell system which are polypeptide producing cells (Pearse 1969)

In the present study the enterochromaffin cells and the Paneth cells were identified by their FIF and no attempts were made to idetify the rare enteroglucan cells which also have been shown to exhibit OPTIF (Unger et al 1966 Polak et al 1971)

VII GENERAL DISCUSSION

Uptake mechanism and secretion

The uptake of dopamine and L-dopa by the Paneth cells of the mouse has been described first by Penttilä and Ahonen (1969) This uptake can be intensified by monoamine oxidase inhibitors nialamide and pargyline hydrochloride, and prevented by cocaine and imipramine (Ahonen and Penttilä 1970 1971) which are inhibitors of the amine pump at the cell membrane level (Muscholl 1961 Berti and Shore 1967 Reiffenstein 1968 Ross et al. 1971) Reserpine promazine amphetamine amitriptyline and nortriptyline have no effect on this uptake (Ahonen and Penttilä 1971) Chlorpromazine is konwn to have a central anticholinergic activity (Cosroe and White 1972) and it liberates adrenaline from the adrenal medulla (Vapaatalo et al. 1969) but has no effect on the dopamine uptake of the Paneth cells of the mouse (Ahonen and Penttilä 1971)

Guanethidine is known to induce chemical sympathectomy (Eränkö and Eränkö 1971 Heath et al. 1972) and it releases catecholamines from the adrenal medulla (Jaanus et al. 1968) It has no effect on the dopamine uptake of the Paneth cells of the mouse (Ahonen and Penttilä 1970 1971) Neither had 6-hydroxydopamine an inhibitory effect on the dopamine uptake but instead it inhibited the secretion of the Paneth cells (Chapter IV) more than vagotomy (Chapter III) which in turn, had an inhibitory affect also on the granule synthesis (Trier et al. 1967) Possible effects of guanethidine on the Paneth cell secretion have not been studied. Noradrenaline is shown to decrease the mesenteric blood flow whereas acetylcholine increases it (Shehadeh et al. 1969) Total bilateral vagotomy decreases also the mucosal blood flow (Padula et al. 1968) Both sympathectomy (Chapter IV) and vagotomy (Chapter III) have inhibitory effect on the secretion of the Paneth cells This effect cannot therefore be explained merely by a decreased mucosal blood flow which may have some effect on the gastric secretion (Jacobson 1969) but it may have some postvagotomy influences

Alpha-metyl-Dopa, noradrenaline alpha-metyl noradrenaline, and adrenaline are also taken up to the secretory granules of the Paneth cells of the mouse but in a much smaller degree than L-dopa or dopamine, and it is not even possible to detect the uptake of alpha-metyl-noradrenaline and adrenaline without monoamine oxidase inhibition (Ahonen and Penttilä 1971) Tyrosine alpha-metyl-tyrosine 5-hydroxytryptamine and 5-hydrotryptophane are not taken up to the Paneth cell granules of the mouse in a detectable degree (Ahonen and Penttilä 1971) The uptake of labelled tryptophan

and cysteine is similar in the Paneth cells and the pancreatic acinar cells (Merzel 1967)

Protein synthesis and amino acid secretion

Five minutes after dopamine injection the strong green fluorescence was seen throughout the whole intestinal mucosa except for the Paneth cells (Chapter V) Five minutes later fluorescent dopamine began to accumulate in the apical secretory granules of the Paneth cells of the mouse At that time the nucleolus of the Paneth cells was occasionally also slightly fluorescent (Chapter IV) The number of the fluorescent granules reached its maximum from 30 minutes to 2 hours after dopamine injection (Chapter III) Fluorescent granules were secreted into the crypt lumen from 2 to 4 hours after injection (Chapter III) Dopamine stimulated the secretion of the Paneth cells significantly (Chapter III and IV) so that the real basal *Paneth cell secretion takes place more slowly than can be seen by dopamine* labelling This secretion speed is however rather similar to that received by labelling the Paneth cell granules with leucine (Trier et al. 1967) The Paneth cell granule synthesis and secretion is also almost identical to the pancreatic acinar cell secretion (Alm et al. 1971 Kramer and Poort 1972 Palade 1972 Wormesley 1972)

According to the present study the Paneth cell granules may contain vasoactive intestinal peptide which has been shown to be secreted by the intestinal mucosa (Håkanson et al. 1972) This possible polypeptide secretion fits well in the assumption that the Paneth cells belong to the APUD cell series (Ahonen and Penttilä 1971) A striking feature of these APUD cells is that most but not all of them, are in glands or tissues which during organogenesis have developed from the primitive gut as for instance stomach pancreas lungs pituitary thyroid and larynx (Langman 1963 Pearse 1968 Rost et al. 1969 Pearse and Polak 1971 Hage 1973) It has been proposed that APUD cells are derived from the neural crest (Pearse and Polak 1971) According to the present and earlier observations (Ahonen and Penttilä 1971 Chapter V and VI) the Paneth cells fulfill all the other criteria of the APUD cells with the exception that their origin is still unknown.

Common origin of enterochromaffin-like cells and Paneth cells

In the glandular part of stomach there are enterochromaffin-like (EC-like) cells which are argyrophil but not argentaffin and which take up L-

dopa like the Paneth cells and like them show ortho-phthalaldehyde induced fluorescence (OPTIF) (Aures and Håkanson 1968 Håkanson et al. 1969 Håkanson et al. 1969 Tjälve 1971) According to the present study the Paneth cells exhibit OPTIF (Chapter VI) probably due to histidine or arginine or both. These two are known to exist in the Paneth cell granules (Merzel 1967) They may be components of the vasoactive intestinal peptide which can also be visualized with the OPTIF method (Håkanson et al. 1972) and as the Paneth cells are the only big OPTIF positive cell group in the intestinum (Chapter VI) the only possibility is that vasoactive intestinal peptide is secreted by the Paneth cells During a severe atrophic gastritis Paneth cells regularly appear in the mucosa of the human stomach (for ref see Otto and Fett 1972) which normally do not contain these cells.

The FIF of both EC-like cells (Håkanson 1970) and Paneth cells after L-dopa depends on intact decarboxylase activity (Chapter V) It has been proposed that the ability to take up amines or their precursors is a common feature of a wide embryologically related cell group (Pearse 1969) thus it is proposed according to the results of the present work (Chapter V and VI) that EC-like cells and Paneth cells may be differentiated from a common anchestor cell type which resembles more the Paneth cells During a severe atrophic gastritis these EC-like cells can possibly be converted into Paneth cells. According to this the Paneth cells may be the least differentiated secretory cell type in the gastrointestinal canal.

Colitis ulcerosa and Paneth cells

Lysozyme activity has been shown in the granule fraction of the Paneth cells (Deckx et al. 1967 Ghoos and Vantrappen 1971) During ulcerative colitis small intestinal Paneth cells resembling cells appear into the human colon (Lauren 1961 Toner 1968 Otto and Fett 1972) It has been shown earlier that secretion of the Paneth cells is accelerated by sympathetic and parasympathetic stimulation (Chapter III and IV) and that parasympathetic stimulation has some influence also on the granule synthesis (Trier et al. 1967) and that both sympathectomy and parasympathectomy inhibit the secretion (Chapter III and IV) It has been proposed that ethiology of the ulcerative colitis may be sacral parasympathetic hyperactivity (Boyd 1961) It has also been shown that colonic motility of the ulcerative colitis patients is decreased (Bloom et al. 1968) The decreased colonic motility may also reflect the increased sympathetic tonus in the colon which is known to have an inhibitory effect on the taenia coli (Lum et al. 1966

Bülbring et al. 1969 Ohkawa et al 1972) Ulcerative colitis patients secrete abnormally great amounts of lysozyme into the feces and ulcerations are often in the taenial region (Boyd 1961) where the number of Paneth cells per surface area of mucosa is greatest (Chapter II) Thus the increased lysozyme secretion during ulcerative colitis may be a reflection of increased sympathetic and/or parasympathetic stimulation on the Paneth cells which in turn may induce during long time dedifferentation of the colonic Paneth cells into the small intestinal stage.

Crypt basement membrane

The crypt basement membrane shows intense FIF after injection of a large dose of dopamine and this fluorescence is similar to that observed in the secretory granules of the Paneth cells (Chapter V) The other epithelial cells in the intestinal crypts have not such dopamine binding ability (Ahonen and Penttilä 1971) After decarboxylase enzyme inhibition and L-dopa injection the crypt basement membrane is also non-fluorescent similarly to the Paneth cell granules (Chapter V) The crypt basement membrane or its dopamine-binding component may be secreted by the Paneth cells in the same way as the epithelial cells (Pierce and Nakane 1972) The basement membrane is possibly composed of a single glycoprotein without hydroxyproline and hydroxylysine (Johnson and Starcher 1972) The core of the Paneth cells granule is also muco or glycoprotein (Selzman and Liebelt 1961 1962 a b Taylor and Flaa 1964)

According to the present work the cell membrane of the Paneth cells seems to be different from the other usual epithelial cells of the intestine at least funtionally because it prevents the passive diffusion of dopamine into the cell (Chapter III IV and V)

Crypt morphology and Paneth cells

It has been shown recently that milk diet increases the number of Paneth cells (Recknagel et al. 1972) Diet lactation and intestinal bacterial flora inhibit the cell migration in the intestinal villi (Koldovsky et al. 1966) and influence also the mucous membrane morphology (Pfeiffer 1968 Herbst and Sunshine 1969 Loehry and Creamer 1969 a, b) Artificial feeding produces precocious development and maturation of the intestinal crypts (Herbst and Sunshine 1969) When the morphology of the ileum and the

jejunum begins to deform the first pathologic step is the formation of intervillous ridges, after which the caudal small intestinum begins to resemble the normal duodenum (Loehry and Creamer 1969 a, b) This "duodenalisation" is the first pathologic step during lactation, intestinal bacterial infection, recovery after methotrexate treatment or during coeliac disease (Loehry and Creamer 1969 a, b) It is interesting to notice that the number of Paneth cells is significantly smaller in the duodenum than in the caudal small intestinum and the first pathologic alterations during malabsorbtion can be detected in the duodenum (Toner 1968) It has been proposed that crypt morphology remains normal as long as there are Paneth cells left in the intestinum but that morphology is disturbed if the Paneth cells are lacking (Creamer 1968) In the present work the crypt basement and villous morphology was disturbed if the secretion of the Paneth cells was inhibited by chemical sympathectomy with 6-hydroxydopamine (Chapter IV) Vagotomy had not so great inhibitory effect on the secretion of the Panet cells and was not able to disturb the crypt morphology Paneth cells appeared in the small intestinum of the mouse at the time when the crypt began to develop (Chapter I) which may support the above hypothesis together with the earlier observations

The effect of the Paneth cells on the crypt morphology can possibly be explained simply by the different mitotic frequencies between the Paneth cells and the other epithelial cells in the crypt. It has been shown that Paneth cells never divide (Deschner 1967 Cheng et al. 1969 Trougton and Trier 1969) whereas the other epithelial cells round them divide rapidly resulting in the Paneth cells remaining between them like at the bottom of a well

MAIN NEW RESULTS AND CONCLUSIONS

1 Dopamine stimulated secretion of the Paneth cells
2. Vagotomy inhibited secretion of the Paneth cells
3 Chemical sympathectomy with 6-hydroxydopamine almost totally prevented secretion of the Paneth cells and caused deformation of the intestinal crypt basement membrane, crypts and villi.
4 Duodenal Paneth cells had significantly more granules than the jejunal ones but both duodenal and jejunal Paneth cells had the same capacity to form and secrete granules.
5 The effect of sympathectomy and vagotomy was greater on the jejunal than the duodenal Paneth cells.

6 Crypt development began at the same time as the appearance of the Paneth cells
7 Paneth cells received their dopamine uptake ability at the age of three weeks which possibly means the beginning of the polypeptide or amino acid secretion.
8 Paneth cells reached their normal adult number and dopamine uptake ability at the age of about 4 weeks
9 The cells at the bottom of the colonic crypts were able to take up dopamine. They were possibly related to small intestinal Paneth cells
10 The crypt basement membrane took up L-dopa but did not show formaldehydeinduced fluorescence after decarboxylase inhibition and L-dopa injection
11 The crypt basement membrane took up dopamine. It may be formed and secreted by the Paneth cells
12 There was ortho-phthalaldehyde induced fluorescence (OPTIF) in the Paneth cell granules and the crypt basement membrane.
13 Cholinergic and adrenergic nerve fibers were in close relationship with the crypt basement membrane but they never penetrated it.
14 Paneth cells were possibly related with the EC-like cells of the stomach.

FIGURES

Figures 1—4 are Thioflavine T fluorescence photographs of sections of the developing mouse fixed with the buffered formaldehyde.

Fig. 1. The cross section of the of the duodenum of a two-weeks-old mouse where fluorescent Paneth cell granules can be seen at the bottom of one crypt (arrow) No fluorescent goblet cells are visible. 250×

Fig. 2. The colon of the two-weeks-old mouse where the fluorescent round goblet cells are visible, and the basal epithelial cells at the bottom of the colonic crypts (colonic Paneth cells) are also fluorescent and not well distinguished from the goblet cells. 250×

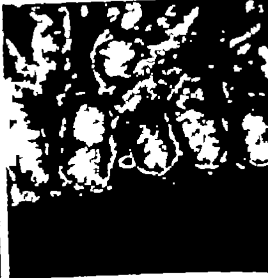
Fig. 3 The jejunum of the four-weeks-old mouse where fluorescent Paneth cells are well visible at the bottom of the crypts. No fluorescent cells can be seen higher up in the crypts or the villi. The number of Paneth cells has reached its normal adult level. 250×

Fig. 4 The ileum of a three-weeks-old mouse where some fluorescent granular Paneth cells are visible. Also some goblet cells can be seen. 250×

Fig. 5 A section of the duodenum of a two-weeks-old mouse fixed with formaldehyde and stained with Best's carmine 2 hours after nialamide and 30 minutes after dopamine injection. The long arrows indicate three Paneth cells, the granules of which are still pale stained. One eosinophil cell can be seen higher up in the crypt (short arrow). White cytoplasmic round nuclear cells in the lamina propria (marked L) possibly represent lymphoid cells. 400×

Fig. 6. A freeze-dried section of the duodenum of the three-weeks-old mouse embedded in paraffin. The section is taken 2 hours after nialamide and 30 minutes after dopamine injection and treated for formaldehyde induced fluorescence according to the standard method. The Paneth cells at the bottom of the crypts are already capable of taking up dopamine. 250×





Figures 7—9 are electron microscopic photographs of the sections of the duodenum of the developing mouse fixed with glutaraldehyde-osmium tetroxide and poststained with lead citrate and uranyl acetate.

Fig. 7 shows the apical part of the duodenal Paneth cell of the mouse with a well developed Golgi Complex (Gol) and a rich rough endoplasmic reticulum (RER) The specific secretory granules have a large halo (SGr) and 5 small electron dense bodies (gra) are visible in the Golgi region. 10.000×

Fig. 8. The basal part of the same cell as in Fig. 7 The cell has a prominent nucleolus (Kro) which is electron dense with one projection downwards and it seems to be continuous with the nuclear envelope on the left side. The cell has numerous free ribosomes in its cytoplasm (rib) and the mitochondria are big and round to oval shaped (mi) The rough endoplasmic reticulum in the upper part of the figure is marked with RER. This Paneth cell is very long and narrow projecting from the basement membrane (BM) up into the cryptal lumen. 10.000×

Fig. 9 An electron microscopic photograph of the duodenum of the four-weeks-old mouse where the nucleolus (Kro) is also prominent. In the nuclear membrane there are some pores (short thick arrow) visible. The mitochondria are big and of different shape (small arrow) The rich rough endoplasmic reticulum has many sac-like dilations (long arrow) Typical apical electron dense secretory granules ar without a halo Some small electron dense bodies (gr) are visible in the Golgi region (Gol) In the middle of the Golgi apparatus there is an area filled with amorphous material (AM) 12.000×


Gol
RER
BM

AM
Gol
Br
Kro
Mi
B

Figures 10—15 are 7 μ thick sections of the colon of the adult mouse fixed with formaldehyde buffered with phosphate.

Fig. 10 shows the section of the colon of the mouse stained with hematoxylin-eosin where it is difficult to identify the colonic epithelial cells at the bottom of the crypt from the goblet cells. 250×

Fig. 11 Section of the colon of the mouse stained with iron hematoxylin where it is possible to differentiate the goblet cells from the epithelial cells at the bottom of the crypts, but not very accurately 400×

Fig. 12. A fluorescence photograph of the colonic section of the mouse stained with Thioflavine T This staining method makes it possible to differentiate the strongly fluorescent goblet cells from the basally not so strongly fluorescent epithelial cells of the crypt. 250×

Fig. 13 A Thioflavine T fluorescence photograph of the colon of the mouse near the taenia coli with more basally located epithelial cells per surface area of mucosa than in the other regions of the colon. These basal epithelial cells are morphologically different from the small intestinal Paneth cells. 250×

Fig. 14 A Thioflavine T fluorescence photograph of the colon of the mouse where the short arrow indicates a colonic basal epithelial cell or colonic Paneth cell with the apical secretory granules which are well visible 250×

Fig. 15 A thioflavine T fluorescence photograph of the normal colon of the mouse where the colonic Paneth cells change into goblet cells in the upper part of the crypt. 250×








Fig. 16. A freeze dried Epon section of the normal colon of the mouse stained with toluidine blue 2 hours after nialamide and 30 minutes after dopamine injection. The short arrow indicates basally located epithelial cells in the crypt or so called colonic Paneth cells which can best be identified by their prominent nucleolus and pale, slightly granular apical cytoplasm. Higher up in the crypt there are many dark stained, distinctly granular cells which seem not to be the usual goblet cells. The number of these granular cells is greater after dopamine injection than in the control group. 400×

Figures 17—20 Formaldehyde-induced fluorescence (FIF) photographs of the freeze-dried Epon sections 2 hours after nialamide and 30 min. after dopamine injection

Fig. 17 Colonic section of the four-weeks-old mouse where green dopamine fluorescence is located in the apical secretory granules of the colonic Paneth cells. Normally there is no catecholamine fluorescence in these cells. In the upper part of the figure there is no catecholamine fluorescence in these cells. In the upper part of the figure to 5HT 250×

Fig. 18. Colonic section of the adult mouse where green fluorescent granules are distinctly visible. 400×

Fig. 19 Fluorescent granules in the apical part of the colonic Paneth cells. 900×

Fig. 20 Green fluorescence in the small granules in the apical part of the cells against the dark cytoplasm. 400×

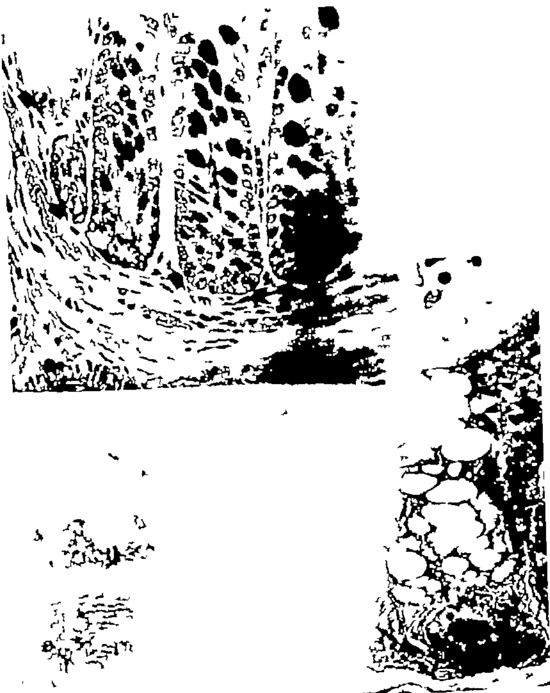


Fig. 21 An electron microscopic picture of the colon of the adult mouse. Basal epithelial cells in the crypt have paler secretion granules (SR) than the small intestinal Paneth cells but have well developed Golgi apparatus (G) and prominent nucleolus (KR) The arrows indicate the other granule type which is smaller and darker in the picture. In the cryptal lumen there are some coliformic bacteria (EC) amongst the secreted material. No real junctions seem to exist between these epithelial cells. 7.500×

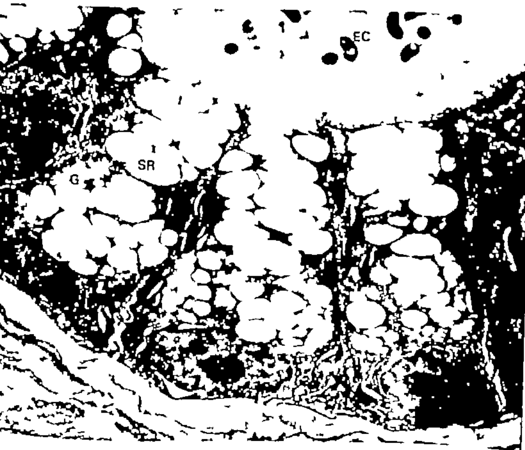

EC
SR
G

Fig. 22. An oil immersion light microscopic photograph of the freeze-dried Epon section, stained with toluidine blue, of the jejunum of the mouse 2 hours after nialamide (500 mg/kg) and 30 minutes after dopamine (160 mg/kg) injection. The section was kept for 2 hours in 60 C 0.1 N HCl solution before staining. The Paneth cell granules *show a weak marked metachromasia. Fig. shows an oblique cross section near* the crypt bottom. 900×

Fig. 23. An oil immersion formaldehyde induced fluorescence (FIF) photograph of the freeze-dried Epon section from the jejunum of the mouse 2 hours after nialamide and *10* minutes after dopamine injection. The other *epithelial cells* and the *lamina* propria are strongly fluorescent. The cytoplasms of the Paneth cells are dark, and in the apical part of the cells there are some Paneth cell granules already visible. 900×

Fig. 24 An oil immersion Thioflavine T fluorescence photograph of the section of the jejunum of the adult mouse fixed *with formaline.* The Paneth *cell granules* are strongly fluorescent and the basement membrane is also visible. 900×

Fig. 25 A FIF photograph of the ileum of the adult mouse 2 hours after nialamide and 30 minutes after dopamine injection. Fluorescent granular Paneth cells are visible in every crypt. The basement *membrane is also fluorescent.* 250×

Fig. 26. An oil immersion light microscopic photograph of the non-specific cholinesterase reaction in duodenum of the adult control mouse. There is positive reaction in a nerve fiber below the crypt basement membrane. The arrow indicates vacuoles near the Paneth cell. The Paneth cell granules are also positive. 1.000×

Fig. 27 An oil immersion photograph of acetylcholinesterase reaction in the duodenum of the adult control mouse. The stained granular nerve fiber (long arrow) is in close contact with the crypt basement membrane (short arrows) in the region of the Paneth cells. 1.200×





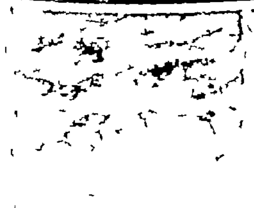
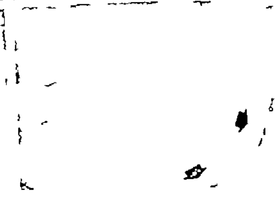



Fig. 28. Acetylcholinesterase reaction in the jejunum of the adult control mouse. Acetylcholinesterase positive nerve fibers are numerous in the muscle layer and one stained granular nerve fiber (arrow) is in close contact with the crypt basement membrane at the site of the Paneth cell. 250×

Fig. 29 An oil immersion photograph showing the section of the jejunum of the vagotomized adult mouse fixed with formaline and stained with Best's carmine. The figure shows the section through the long axis of the crypts. Seven big Paneth cells are filled with the typical secretory granules with a big halo. One lymphoid cell is near the basement membrane (arrow) outside it. 900×

Fig. 30 The number of Paneth cell granules per cell amounts to a total of 600 Paneth cells in the mid-duodenum and mid-jejunum 1 hour and 30 minutes after nialamide (500 mg/kg) and 30 minutes after dopamine (160 mg/kg) injection. The group treated with dopamine differs significantly from the control group ($p < 0.001$) The granules were calculated in the vagotomized group 24 hours after vagotomy and that, too, differs significantly from the control group According to these calculations vagotomy inhibited and dopamine stimulated the secretion significantly

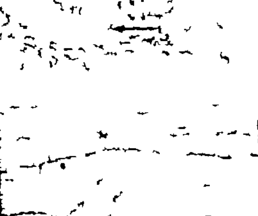




Fig 30

gran/PC

duodenum

jejunum

16
14
12
10
8
6
4
2

control
dopamine
vagotomy
control
pamine
vagotomy

Fig. 31 The distribution of the Paneth cells of the mouse are given as a function of their number of granules. The same experiment groups are presented as in Fig. 30 The dotted line marks the average number of granules per cell. The dopamine and the vagotomized group both *differ significantly from the control group.*

Fig. 32. An oil immersion photograph showing the section of the jejunum of the adult mouse fixed with formaldehyde and stained with Best's carmine 2 days after 6-hydroxydopamine injection. The whit cytoplasmic cells (L) are lymphoid cells. The Paneth cells at the bottom of the crypt are *filled with big granules.* 900×

duodenum vagotomy jejunum

No of PC

150 150

100 100

50 50

5 10 15 20 25 gran/PC 1 5 10 15 20 25

duodenum dopamine jejunum

No of PC

150 150

100 100

50 50

5 10 15 20 25 gran/PC 1 5 10 15 20 25

duodenum controls jejunum

No of PC

150 150

100 100

50 50

5 10 15 20 25 gran/PC 1 5 10 15 20 25



Fig. 32



Fig. 34



Fig 36



Figures 33—38 FIF photographs of freeze-dried sections of the jejunum of the mouse embedded in Epon. The samples were taken 2 hours after nialamide (500 mg/kg) and 5 min. (Fig. 33) 15 min. (Fig. 34 35 and 36) and 30 min. (Fig. 37 and 38) after dopamine injection (160 mg/kg) The Epon sections in Figs. 33—36 are 2 μ thick and in Figs. 37 and 38 5 μ thick. All the mice in the Figs. 33—36 and 38 were given 6-hydroxydopamine (50 mg/kg) intraperitoneally 2 days earlier

In Fig. 33 the cytoplasm of the Paneth cells is dark (arrow) against the other strongly fluorescent epithelial cells. 900×

In Fig. 34 a short arrow indicates a migrating fluorescent cell between the epithelial cells. Similar fluorescent granular cells can also be seen in the lamina propria (medium sized arrow) These moving granular cells are leukocytes, probably eosinophil leukocytes. Two long arrows indicate Paneth cells with dark stained cytoplasm and a slightly fluorescent nuclear membrane. Inside the nucleus there are slightly fluorescent particles visible which ar probably nucleoli. 900×

In Fig 35 an arrow indicates a Paneth cell with fluorescent granular material in its Golgi region. The nuclear membrane is slightly fluorescent and inside the nucleus there is one great fluorescent particle visible, probably nucleolus. 900×

In Fig. 36 an asterisk indicates a fluorescent cell, probably an eosinophil leukocyte, in the epithelium of the jejunum of the mouse. Outside the fluorescent cell membrane of this eosinophil cell there is fluorescent granular material in the Golgi region of the Paneth cell. 900×

In Fig. 37 two dark cytoplasmic Paneth cells are distinctly visible with their fluorescent apical cytoplasmic granules against the green fluorescent background. 900×

In Fig. 38 the crypts of Lieberkühn are cross sectioned at their mid-part. Strongly fluorescent cells are visible in the lamina propria outside the crypt basement membrane (arrow) 400×



Fig. 39

Fig. 39 An oil immersion photograph showing the section of the duodenum of the mouse fixed with formaldehyde and stained with Best's carmine two weeks after 6-hydroxydopamine injection (50 mg/kg) Three long arrows indicate the crypts with some Paneth cells at their bottom. Smallest arrow indicates the basement membrane of the crypts which is deformed and loosened from the epithelial cells of the crypt. In these deformed crypts there are no Paneth cells visible. The big arrows indicate the slightly deformed villi of the duodenum of the mouse. There are often great accumulations of white stained material (asterisk) below the deformed basement membrane 250 X

gran / PC
duodenum
jejunum
18
16
14
12
10
8
6
4
2
control
dopamine
sympath+dopamine
sympathectomy
control
dopamine
sympath+dopamin.l
sympathectomy

Fig. 40

duodenum
sympathectomy+dopamine
jejunum
No of PC No of PC
150 150
100 100
50 50
gran/PC 1 5 10 15 20 25 30 1 5 10 15 20 25 30 gran/PC
duodenum
sympathectomy
jejunum
No of PC No of PC
150 150
100 100
50 50
gran/PC 1 5 10 15 20 25 30 1 5 10 15 20 25 30 gran/PC
duodenum
dopamine
jejunum
No of PC No of PC
150 150
100 100
50 50
gran/PC 1 5 10 15 20 25 30 1 5 10 15 20 25 30 gran/PC
duodenum
control
jejunum
No of PC No of PC
150 150
100 100
50
gran/PC 1 5 15 20 5 10 15

Fig. 41

Fig. 42. An electron microscopic photograph of the jejunum of the mouse 1 week after sympathectomy. The mouse received nialamide 2 hours and dopamine 30 minutes before being killed. The microvilli of the Paneth cell are visible at the upper part of the figure. Large secretory granules with an irregular halo are visible. Between two Paneth cells there is one long processus probably belonging to a leukocyte that penetrates into the crypt through the intercellular space. The processus is full of big mitochondria and round grey granules. 15,000×

Figures 43—47 are formaldehyde induced fluorescence photographs (FIF) of the jejunum of the mouse 2 hours ... mide injection.

Fig. 43. The situation ... after dopamine injection. The cytoplasm of the epithelial cells exc... cells are diffusely fluorescent. Fluorescent material begins to accumulate in ... granules of the Paneth cells. The nucleolus of one Paneth cell (arrow) ... dopamine. The basement membrane and the lamina propria are strongly ... ×

Fig. 44. The s... nutes after dopamine injection. The basement membrane is strongly fluo... so are also the nerve fibers in the lamina propria. On the left there is o... posed enterochromaffin cell. Three Paneth cells are at the bottom of the ... apical cytoplasmic granules. Between the enterochromaffin and the Paneth cel... one cell, the cytoplasm of which is diffusely fluorescent and with four small ... granules in its cytoplasm. 900×

Fig. 45. The situation 10 minutes after dopamine injection. The mouse was given 6-hydroxydopamine 1 week earlier. Five dark cytoplasmic Paneth cells are visible and dopamine begins to accumulate in the secretory granules. Between the two basal Paneth cells there is one strongly fluorescent cell. The space between the two Paneth cells is fluorescent and may belong to leukocytes that penetrate into the crypt lumen between the Paneth cells. A possibly similar processus is visible by electron microscopy in Fig. 42. The basement membrane is strongly fluorescent (star) and is loosened from the also fluorescent cell membrane of the Paneth cell. 900×

Fig. 46. The situation 3 hours after Ro 4602 and 2 hours and 30 minutes after L-dopa injection. Three Paneth cells at the bottom of the crypt are totally negative. There is one partly emptied enterochromaffin cell (EC) between the Paneth cells. At the site of the Paneth cells and the enterochromaffin cell the basement membrane is negative. This negatively stained basement membrane makes a distinct contrast to the membranes in Figs. 43, 44 and 45. In those experiments decarboxylase inhibitor was not used. 1,000×

Fig. 47. The same experiment as in Fig. 46 but the picture is taken near the tip of the villus. One green fluorescent mast cell (MC) and three green fluorescent eosinophil cells (E) are visible. The eosinophil cells have great fluorescent granules in their cytoplasm. In the epithelial lining there are some small weakly fluorescent granules visible. 900×

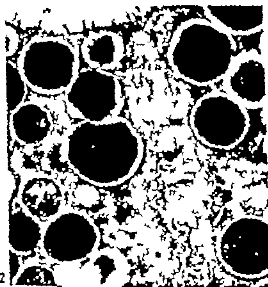


Fig. 42





Fig. 44



Fig. 49



Fig. 46

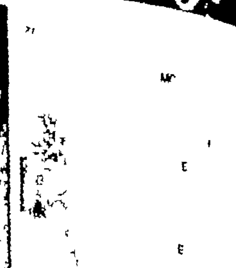


Fig. 51

Fig. 48. FIF in the jejunum of the adult mouse. The mouse received nialamide (500 mg/kg) 3 hours and dopamine (160 mg/kg) 30 minutes before the killing. Fluorescent granules of the Paneth cells are visible. Green fluorescence is seen also in the basement membrane of the crypt. 200×

Fig. 49 FIF in the colon of the four weeks-old mouse. Drugs were given as in Fig. 48. Green fluorescent Paneth cell granules are visible against the dark cytoplasm. 250×

Fig. 50. OPTIF in the jejunum of the mouse. Drugs were given as in Fig. 48. The Epon section was moistened with water and one drop of 1% OPT in xylene was put on the slide. Fluorescence appeared in 10 minutes in the Paneth cells (P) and also in some other granular cells in the crypt lumen. One fluorescent cell in the lamina propria is a mast cell (arrow) and there is an enterochromaffin cell in the adjacent crypt that is also fluorescent with OPT (asterisk) The nerve fibers are non-fluorescent after moistening with water. 250×

Fig. 51 An OPTIF photograph of the ileum of the mouse treated as in Fig. 48. Nialamide was given 3 hours and dopamine 1 hour before killing. The Paneth cell (P) granules are distinctly fluorescent. More eosinophil fluorescent cells and higher up in the crypt as compared with Fig. 50 are visible. 250×



Fig. 48



Fig. 49



Fig. 50



Fig. 51

Fig. 52. OPTIF in the jejunum of the mouse. Nialamide was given 3 hours and dopamine 45 minutes before killing. More Paneth cell granules are visible as compared with FIF indicating that not all the granules become visible with FIF after a single dopamine injection. 250×

Fig. 53. OPTIF in the jejunum of the adult mouse. The mouse fasted two days after which it was allowed to eat 5 hours standard food, received nialamide 4 hours and dopamine 3 hours and 30 minutes before killing. Paneth cell granules are visible with OPTIF. Numerous cells in the lamina propria and submucosa are visible with OPTIF. The basement membrane is also fluorescent (arrow). 250×



Fig. 52



53

REFERENCES

ABERG, G and O ERÄNKÖ Localisation of noradrenaline and acetylcholinesterase in the taenia of the guinea pig caecum. Acta Phys. Scand. 1967 69 383—384

AHONEN, A., Effect of pilocarpine on the accumulation of dopamine in the Paneth cells of the mouse. Scand. J Clin. Lab. Invest. 1970. 25 suppl. 113 17

AHONEN A. and A. PENTTILÄ Accumulation of catecholamines in the mouse Paneth cells. Scand. J Clin. Lab. Invest. 1969 23 suppl. 108 34

AHONEN A. and A. PENTTILÄ Effect of some drugs on the accumulation of dopamine in the mouse Paneth cells. IX International Congress of Anatomists. Leningrad. 1970.

AHONEN A. and A. PENTTILÄ, Effect of some drugs on the uptake of monoamines and their precursors in the mouse Paneth cells. Acta Physiol. Scand. 1971 82. 59—69

ALM, P EKHOLM, R. and L. E. ERICSSON Rate of appearance of metabolites in the rat pancreatic juice after administration of ^{14}C-L-dopa. Comm. Departm. Anat. University of Lund. 1971 No 8.

ANDERSSON, R., Role of cyclic AMP and Ca++ in the metabolic and relaxing effects of catecholamines in intestinal smooth muscle. Acta Physiol. Scand. 1972. 85 312—22.

AURES, D and HÅKANSSON R., Amine formation in polypeptide-producing endocrine cells of the digestive tract. Eur. J Pharmacol. 1968. 3 316—321.

BÄCK, O Studies on the lymphocytes in the intestinal epithelium of the chicken. Intern. Arch. Allerg. Appl. Immunol. 1970 39 342—351

BÄCK, O Studies on the lymphocytes in the intestinal epithelium of the chicken. I. Ontogeny Acta Path. microbiol. Scand. Sect. A. 1972. 80. 84—90.

BEHNKE, O and H. MOE, An electron microscopic study of mature and differentiating Paneth cells in the rat especially of their endoplasmic reticulum and lysosomes. J Cell. Biol. 1964 22. 633—652.

BERTI, F and P A. SHORE, A kinetic analysis of drugs that inhibit the adrenergic neuronal membrane amine pump Biochem. Pharmacol. 1967 16. 2091—2094

BLOOM, A. A., LOPRESTI, PH. and J T FARRAR, Motility of the intact human colon. Gastroenterology 1968. 54 232—240

BOWER, D and C. G CHADWIN Demonstration of Paneth cell granules using Naphthalene black. J Clin. Path. 1968. 21 107

BOYD W Textbook of Pathology 7 ed. by Henry Kimpton. 1961 London.

BROWNING T H. and J S TRIER, Organ culture of mucosal biopsies of human small intestine. J Clin. Invest. 1969 48 1423—1432.

BOLBRING E. and T TOMITA, Supression of spontaneous spike generation by catecholamines in the smooth muscle of the guinea pig taenia coli. Proc. Roy Soc. B. 1969 172. 103—119

BURNS J and R. WHITEHEAD Staining of Paneth cells with Thioflavine T Nature 1966. 212. 769—771.

CALDARERA, C. M., GIORGI P P and A. CASTI The effect of noradrenaline on polyamine and RNA synthesis in the chick embryo J Endrocrinol. 1970 46. 115—116.

CHENG H., MERZEL, J and A. C. LEBLOND Renewal of Paneth cells in the small intestine of the mouse. Am. J Anat. 1969 126. 507—525

CONSOLO S GARATTINI S LADINSKY H. and H. THOENEN Effect of chemical sympathectomy on the content of acetylcholine, choline and choline acetyltransferase activity in the cat spleen and iris. J Physiol. 1972. 220 639—646.

CONSROE, P F and R. P WHITE, Effects of haloperidol and chlorpromazine on central adrenergic and cholinergic mechanisms in rabbits. Arch. Intern. Pharmacodyn. Therap 1972. 198. 67—75

COSTA, M. and G GABELLA, L'innervation adrénergique du canal alimentaire. Bulletin de l Association des Anatomistes. 1967 52. 351—356.

CREAMER, B., Paneth-cell function. Lancet. 1967 Feb. 11 314—316.

DECASTRO M., SASSO W D and F A. SAAD Preliminary observations of the Paneth cells of the Tamandua tetradactyla. Acta Anat. 1952. 38 345—352.

DECKX, R. J VANTRAPPEN G R. and M. M PAREIN Localization of lysozyme activity in a Paneth cell granule fraction. Biochem. Biophys. Acta. 1967 139 204—207

DESCHNER E. E. Observations on the Paneth cell in human ileum. Exp. Cell. Res. 1967 47 624—628

EHINGER, B., HAKANSSON R., OWMAN Ch. and SPORRONG B., Histochemical demonstration of histamine in paraffin sections by fluorescence method. Biochem. Pharmacol. 1968. 17 1997—1998.

EHINGER, B and THUNBERG R., Induction of fluorescence in histamine containing cells. Acta Histochemica. 1967 47 116—122.

EISENHUTH, von I and G GEYER, Zytologische und Zytochemische Untersuchungen über Verenderungen am Dünndarmepithel der Albinomaus durch chronische Be handlung mit Antimetabiliten Riboazauracil und Äthionin. Acta Histochemica. 1966. 25 321—328

ELFIN L-G Ultrastructural studies on the synaptology of the inferior mesenteric ganglion of the cat. I Observations on the cell surface of the postganglionic perikarya. J Ultrastructural Res. 1971 a. 37 411—425

ELFIN L-G II Specialized serial neuronal contacts between preganglionic end fibers. J Ultrastruct. Res. 1971 b. 37 426—431

ELFIN L-G III The structure and distribution of the axodendritic and dendrodentritic contacts. J Ultrastruct. Res. 1971 a. 37 432—448.

EMAS S., Vagal influences on gastric acid secretion. Scand. J Gastroenterol. 1973 8. 1—4

ENERBÄCK, L., Detection of histamine in mast cells by o-phthalaldehyde reaction after liquid fixation J Histochem. Cytochem. 1969 17 757—759

ERÄNKO O The practical histochemical demonstration of catecholamines by formaldehyde induced fluorescence. J Roy Micr Soc. 1967 87 259—276.

ERÄNKO L. and O ERÄNKO Effect of guanethidine on nerve cells and small intensely fluorescent cells in sympathetic ganglia of newborn and adult rats. Acta Pharmacol. Toxikol. 1971 30 403—416.

ERLANDSEN S L. and D G CHASE, Paneth cell function. Phagocytosis and intracellular digestion of intestinal micro-organisms. I Hexamita muris. J Ultrastruct. Res. 1972, a 41 296—318.

ERLANDSEN S L. and D G CHASE, Paneth cell function Phagocytosis and intracellular digestion of intestinal micro-organisms. II. J Ultrastruct. Res. 1972, b. 41 319—333

FINCH, L. and G. D. H. LEACH Effect of 6-hydroxydopamine on the perfused rat mesentery preparation. J Pharm. Pharmacol. 1970 22. 535
GABELLA, G. and M. COSTA, Adrenergic fibers in the mucous membrane of guinea pig alimentary tract. Experientia. 1968. 24. 706—707
GENT A. E. and B. CREAMER Paneth cell secretion. Digestion. 1972. 7 1—12.
GEYER, G. von, Eine histochemische Methode zur differenzierten Darstellung von Kohlenhydrat und Protein Bestandteilen in den Prosecretgranula Panethschen Körnzellen der Maus. Acta Histochemica 1966. 25 55—57
GEYER, G und W LINSS Histochemie und Feinstructur der Prosecretgranula in den Panethschen Zellen der Maus. Naturwissenschaften 1965 52. 14—15
GHOOS Y and G VANTRAPPEN The cytochemical localization of lysozyme in Paneth cell granules. Histochem. J 1971 3 175—178
GOSLING J A. and J S. DIXON The effect of 6-hydroxydopamine on nerves in the rat upper urinary tract. J Cell. Sci. 1972. 10 197—209
HAGE, E., Amine handling properties of APUD- cells in the bronchial epithelium of human fetuses and in the epithelium of the main bronchi of human adults.
HAKANSON R., JOHANSSON H and A. L. RÖNNBERG OPT-induced fluorescence of glucagon and secretin. Acta Physiol. Scand 1971. 83 427—429
HAKANSON R., LINDSTRAND K. and NORDGREN L. and Ch. OWMAN, Histamine containing epithelial cells in the rat stomach A possible storage site for intrinsic factor Eur J Pharmacol. 1969 8. 315—325
HAKANSON R., OWMAN Ch. and N-O SJÖBERG Three different systems of monoamine storing cells in the gastrointestinal tract of fetal and neonatal rats. Acta Physiol. Scand. 1969 75 213—220
HAKANSON R., OWMAN Ch. and F SUNDLER, O-phthalaldehyde (OPT) A sensitive detection reagent for glucagon, secretin and VIP (vasoactive intestinal peptide) J Histochem. Cytochem. 1972. 20 138—140
HALBHUBER, K-J STIBENZ, H J and U HALBHUBER, Autoradiographische Untersuchungen über die Verteilung einiger Metallisotopen im Darm von Laboratoriumstieren. Ein Beitrag zur Ausscheidungsfunktion der Panethschen Körnzellen. Acta Histochemica. 1970 35 307—319
HALBHUBER, K J von., STIBENZ, H. J and G GEYER, Autoradiographische Untersuchungen über die Synthese des Prosekrets in den Panethschen Körnzellen des Duodenums der albinomaus. Acta Histochemica. 1972. 42. 143—156.
HALLY A. D The fine structure of the Paneth cell. J Anat. 1958. 92. 268—277
HAMPTON J Effects of fixation on the morphology of Paneth cell granules. Stain. Technol. 1965 40 283—291.
HEATH, J W EVANS B K. and B. J GANNON Degeneration of adrenergic neurones following guanethidine treatment An ultrastructural study Virchows Arch. Abt. B Zellpath. 1972. 11 182—197
HERBST J J and P SUNSHINE, Postnatal development of the small intestine of the rat. Pediat. Res. 1969 3 27—33
HIRSCHOWITZ, B. I HUTCHISON G and G SACHS Kinetics of atropine inhibition of histamine-stimulated gastric secretion in the dog. Am. J Physiol. 1972, 222. 1316—1321
HOKFELT T JONSSON G and Ch. SACHS Fine structure and fluorescence morphology of adrenergic nerves after 6-hydroxydopamine in vivo and in vitro. Z. Zellforsch. 1972. 131 529—543

JAANUS S D MIELE, E. and R. P RUBIN The action of guanethidine on the adrenal medulla of the cat. J Pharmacol. Chemotherap. 1968, 33 560—569
JACOBSSON E. D The blood supply of the stomach. Surgical Clinics of North America. 1969 49 559—564
JOHNSON L. D and B. C. STARCHER, Epithelial basement membranes The isolation and identification of a soluble component. Biochem. Biophys. Acta 1972. 290. 158—167
KALINER, M., ORANGE, R. E. and K. F AUSTEN Immunologic release of histamin and slow reacting substance of anaphylaxis from human lung. J Exp. Med. 1972. 136. 556—567
KANERVA, L. and A. HERVONEN Axo-axonal relations in the autonomic nerves of the rabbit myometrium. IV Congress Histochem. Cytochem. 1972. Kyoto.p. 515—516.
KOELLE, G B The elimination of enzymatic diffusion artifacts in the histochemical localization of cholinesterases and a survey of their cellular distributions. J Pharmacol. exp. Therap. 1951 103 153—171
KOLDOVSKY O SUNSHINE, P and N KRETCHMER, Cellular migration of intestinal epithelia in suckling and weaned rats. Nature. 1966. 212. 1389—1390
KOSTERLIZ, H. W and G M. LEEDS In Catecholamines. Handb. Exp. Pharm. XXXIII 1972. ed Blaschko and Muscholl. Springer—Verlag. Berlin—Heidelberg—New York.p. 762—812.
KRAMER, M. F and C. POORT Unstimulated secretion of protein from rat exocrine pancreas cells. J Cell. Biol. 1972. 52. 147—158
KUROSUMI, K., Electron microscopic analysis of the secretion mechanism. Internat. Rev Cytol. 1961 11 1
KUZMICHEVA, N A., ORLOVA, S. A., RODINOV V M., BOLSHAKOVA, T D LUKICHEVA, T D MENSHIKOV V V FROLOV E. P and U N YARGIN Hyposensitivity of smooth muscle to noradrenaline caused by sympathetic hyper innervation. Nature. 1969 224 385
LANGMAN J Medical embryology William & Wilkins Company Baltimore. 1963
LAUREN P The structure and secretion in cancer With reference to benign epithelial tumors of the bowel. Acta Path. microbiol. Scand. 1961 suppl. 152.
LAUREN P and T E. SORVARI Staining of Paneth cells with Best's carmine after metylation. Stain. Technol. 1967 42. 311—315
LICK R. F von., HALARIS A., BALSER, D HART W and I KLEMPA, Zur Ultrastructur der Schleimhautinnervation des Magens. Zeitschrift. Gastroenterol 1968 6. No 5 342—347
LOEHRY C. A. and B. CREAMER, Three dimensional structure of the human small intestinal mucosa in health and disease. GUT 10 6—12.
LOEHRY C. A. and B. CREAMER Three dimensional structure of the rat small intestinal mucosa related to mucosal dynamics. I. Mucosal structure and dynamics after the administration of methotrexate. II. Mucosal structure and dynamics in the lactating rat. III Mucosal structure and dynamics in the rat infested with the nematode Nippostrongylus brasiliensis. GUT 1969 10 112—120.
LUM, D K. B., KERMANI M. H. and R. D HEILMAN Intestinal relaxation produced by sympathomimetic amines in the isolated rabbit jejunum selective inhibition by adrenergic blocking agents and by cold storage. J Pharmacol. exp. Therap 1966 154 463—471

MERZEL, J Some histophysiological aspects of Paneth s cells of mice as shown by histochemical and radioautographic studies. Acta Anatomica 1967 66. 603—630.
MERZEL, J and C. P LEBLOND Origin and renewal of goblet cells in the epithelium of the mouse small intestine. Am. J Anat. 1969 124 281—306
MIRKIN B. L., Onthogenesis of the adrenergic nervous system. Function and pharmacologic implications. Fed. Proc. 1972. 31. 65—73
MORI, T M. J FUJIWARA, M. and K. SHIMAMOTO Fluorescence histochemical demonstration of adrenergic nerve fibers in the vagus nerve of the cats and dogs. Jap. J Pharmacol. 1968 18. 285—293
MUSCHOLL, E., Effect of cocaine and related drugs on the uptake of noradrenaline by heart and spleen. Brit. J Pharmacol. 1961 16. 352—359
NAKANISHI, H. and H. TAKEDA, The possibility that adenosine triphosphate is an excitatory transmitter in guinea pig seminal vesicle. Jap. J Pharmacol. 1972. 22. 269—270.
OHKAWA, H. and C. L. PROSSER, Function of neurones in enteric plexuses in cat intestine. Am. J Physiol. 1972. 222. 1420—1426.
OKUN, M., ELDSTEIN L., OR, N HAMADA, G and B. DONELLAN Histochemical studies of conversion of thyroxine and dopamine to melanin mediated by mammalian peroxidase. Life Sciences. II. 1970 9 491—505
OTTO H. F and R. FETT Zur Orthologie und Pathologie Panethscher Körnzellen. Virchows arch. Abt. A. Path. Anat. 1972. 356. 187—206
OTTO H. F and WEITZ, H., Electronmicroscopische Untersuchung an Paneth- Zellen der Ratte unter Zincarmer Diät. Beitr Path. 1972. 145 336—349
PADULA, R. T NOBLE, P H. and R. C. CAMISHION Vascularity of the mucosa of the small intestine after vagotomy and splanchiectomy Surg. Gyn. Obstetr 1968 127 41—48.
PALADE, G E., Synthesis, transport and discharge of secretory proteins. IV International Congress Histochem. Cytochem. 1972. Kyoto. p. 69—70.
PALMER, G C., Increased cyclic-AMP response to norepinephrine in the rat brain following 6-hydroxydopamine. Life. Sci. Physiol. Pharmacol. 1972. 11 145—149
PEARSE, A. G E., The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic physiologic and Pathologic implications of the concept. J Histochem. Cytochem. 1969 17 303—313
PEARSE, A. G E. and J M. POLAK, Cytochemical evidence for neural crest origin of mammalian ultimobranchial C cells. Histochemie. 1971. 27 96—102.
PENTTILÄ, A. and A. AHONEN Binding of 1—3 4-dihydroxyphenylalanine and dopamine in cytoplasmic granules of Paneth cells. Experientia. 1969 25 70—72.
PFEIFFER, C. J Intestinal villous morphology Clinical Nutrition. 1968 53 215—221
PICK, J DE LEMOS, C. and A. CIANELLA, Fine structure of nerve terminals in the human gut. Anat. Rec. 1967 159 131—146.
PIERCE, G B and P K. NAKANE, Basement membrane: Synthesis and role in disease. IV International Congress Histochem. Cytochem. 1972. Kyoto. p. 125—126.
PITHA, J The fine structure of membraneous inclusions in the Paneth cells of rabbit. Z. Zellforsch. 1968. 90. 563—569
POLAK, J M., COULLING I., BLOOM, S. and A. G E. PEARSE, Immunofluorescent localisation of secretin and enteroglucagon in human intestinal mucosa. Scand. J Gastroenterol. 1971. 6. 739—744

RAUANHEIMO L. and O ERÄNKÖ Simultaneous histochemical demonstration of acetylcholinesterase and noradrenaline in autonomic nerve fibers. Scand. J Clin. Lab. Invest. 1968 suppl. 101. 44—45

RECKNAGEL, I von. GEYER, G and K-J HALBHUBER, Quantitative Untersuchungen über die Entwicklund der Paneth- Zell-Population im Dünndarm der Maus bei ausalleinaliber Milchernährung. Anat. Anz. 1972. 132. 523—529

REIFFENSTEIN R. J Effects of cocaine on the rate of concentration to noradrenaline in the cat spleen strip mode of action of cocaine. Br J Pharmacol. Chemother. 1968. 32. 591—597

RIECKEN E. O and A. G E. PEARSE Histochemical study on the Paneth cell in the rat. GUT 1966. 7 86—93

ROSS S B., RENYI A. L. and S-O ÖGREN A comparison of the inhibitory activities of iprindole and imipramine on the uptake of 5-hydroxytryptamine and noradrenaline in brain slices. Life. Sci. I. 1971 10 1267—1277

ROST F W D POLAK, J M. and A. G E. PEARSE, The melanocyte: Its cytochemical and immunological relationship to cells of endocrine polypeptide (APUD) series. Virchows Arch. Abt. B Zellpath. 1969 4 93—101

SCHAAF P von. und WENZEL, U D Über Vorkommen und Histochemische Klassifizierung von Panethschen Körnzellen bei einigen Säugerspecies. Anat. Anz. 1970 126. 527—530

SELZMAN H. M. and R. A. LIEBELT A cytochemical analysis of Paneth cell secretion in the mouse. Anat. Rec. 1961 140 17—22.

SELZMAN H. M. and R. A LIEBELT Paneth cell granule of mouse intestine. J Cell. Biol. 1962 a. 15 136—139

SELZMAN H. M. and R. A. LIEBELT Localization of sulphydryl and disulphide groups of protein in the Paneth cell secretion of the mouse intestine. J Histochem. Cytochem. 1962 b 10 106.

SHEHADEH, Z., PRICE, W E. and E. D JACOBSON Effects of vasoactive agents on intestinal blood flow and motility in the dog. Am. J Physiol. 1969 216. 386—392.

SHELLEY W B. and S OHMAN Stain for histamine in mast cells in fixed tissues. Histochemie. 1968. 15 287—289

SHELLEY W B., OHMAN S. and H. M. PARNES Mast cell stain for histamine in freeze dried embedded tissue. J Histochem. Cytochem. 1968 16. 433—439

SHIBATA, S KUCHII M. and K. KURAHASHI, The supersensitivity of isolated rabbit aortic strips produced by 6-hydroxydopamine. Eur J Pharmacol. 1972. 18. 271—280.

SHORE, P A., BURKHALTER, A. and U H. COHN A method for fluorometric assay of histamine in tissues. J Pharmacol. Exp. Therap 1959 127 182.

SILVA D G FARRELL, K. E. and G C. SMITH, Ultrastructural and histochemical studies on the innervation of the mucous membrane of the mouse colon. Anat. Rec. 1968 162. 157—176.

SMYTHIES J R., The adenylcyclase complex. A model of its mechanism of action in relation to the α- and β-adrenergic receptors. Eur J Pharmacol. 1972. 19 18—24

SPERSCHNEIDER, H. von., GEYER, G and K-J HALBHUBER, Autoradiographische Untersuchungen über die Entwicklung von Panethschen Zellen im Dünndarm der Maus. Acta Histochemica. 1972. 44 196—202.

STANLEY M. W and J S. TRIER, Morphologic heterogenity of the mouse Paneth cell granules before and after secretory stimulation. Am. J Anat. 1965 117 365—384
TACCA, M. del., SOLDANI, G SELLI, M. and A. CREMA Action of catecholamines on release of acetylcholine from human taenia coli Eur J Pharmacol. 1970 9 80—84.
TAKAGI, K., TAKAYANAGI I. and A. TOMITA, Actions of dibutyryl cyclic adenosine monophosphate, papaverine and isoprenaline on the intestinal smooth muscle. Japan. J Pharmacol. 1971 21 477—483
TAKAYA, K., A new fluorescent stain with o-phthalaldehyde for A cells of pancreatic islets. J Histochem. Cytochem. 1970 18. 178—186.
TAYLOR, J J and FLAA R. C., Histochemical analysis of Paneth cell granules in rats. Arch. Path. 1964. 77 278—285
THOENEN, H. and J P TRANZER, Chemical sympathectomy by selective destruction of adrenergic nerve endings with 6-hydroxydopamine. Nayn.-Schmiedbergs Arch. Pharmak. u. exp. Path. 1968 261 271—288.
TJALVE, H Catechol- and indoleamines in some endocrine cell systems. Acta Physiol. Scand. 1971 suppl. 360.
TONER, P G., Cytology of intestinal epithelial cells. Internat. Rev Cytol. 1968 24 233—343.
TRIER, J S., LORENZSON V and K. GROEHLER, Pattern of secretion of Paneth cells of the small intestine of mice. Gastroenterology 1967 53 240—9
TROUGHTON W D., and J S TRIER, Paneth and goblet cell renewal in mouse duodenal crypts. J Cell. Biol. 1969 41 251—267
UNGER, R. H., KETTERER and A. M. EISENTRAUT Distribution of immunoassayable glucagon in gastrointestinal tissues. Metabolism 1966. 15 865
URETSKY N. J and L. L. IVERSEN Effects of 6-hydroxydopamine on noradrenaline containing neurons in the rat brain. Nature. 1969 221 557—559
VAPAATALO H. I IDÄNPÄÄ-HEIKKILÄ, J E., NEUVONEN P J and M. K. PAASONEN Effects of prolonged chlorpromazine treatment on the rat intestine. Acta Pharmacol. toxicol. 1969 27 262—268.
WORMSLEY K. G The effect of vagotomy on the human pancreatic response to direct and indirect stimulation. Scand. J Gastroenterol. 1972. 7 85—91
OHMAN, S. and W B. SHELLEY o-phthalaldehyde staining of coiled and uncoiled intraepidermal sweat ducts. J Investig. Dermatol. 1969 53. 29—33

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 399

OXYGEN TRANSPORT DURING EXERCISE IN HUMAN SUBJECTS

by

Lars Hermansen

OSLO 197[illegible]

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 399

From the Institute of Work Physiology, Oslo Norway

OXYGEN TRANSPORT DURING EXERCISE IN HUMAN SUBJECTS

by

LARS HERMANSEN

OSLO 1973

The present monograph is based on investigations carried out at the Institute of Work Physiology Oslo Norway the Department of Physiology Gymnastik och idrottshögskolan, Stockholm, Sweden and the Institute of Hygiene University of Oslo Oslo Norway Except for some unpublished results, this monograph includes results which have previously been reported in the following publications

I HERMANSEN LARS and BENGT SALTIN Oxygen uptake during maximal treadmill and bicycle exercise.
J.appl.Physiol. 1969 *26*. 31–37

II HERMANSEN LARS BJÖRN EKBLOM and BENGT SALTIN Cardiac output during submaximal and maximal treadmill and bicycle exercise.
J appl.Physiol. 1970 *29* 82–86

III VELLAR, ODD D and LARS HERMANSEN Physical performance and hematological parameters. With special reference to hemoglobin and maximal oxygen uptake.
Acta med.Scand. 1971 *189* *Suppl.* *522*. 1–40

IV HERMANSEN LARS and MILENA WACHTLOVA, Capillary density of skeletal muscle in well-trained and untrained men
J appl.Physiol. 1971 *30*. 860–863

In the text these publications will be referred to by their Roman numerals.

Methods and Procedures 38
Results 40
Cross-sectional analysis 40
Longitudinal analysis 44
Selected cases of iron deficiency anemia 44
Comments 46

CHAPTER V

CAPILLARY DENSITY OF SKELETAL MUSCLE AND MAXIMAL OXYGEN UPTAKE 47
Previous Investigations 47
Subjects 47
Methods and Procedures 48
Results 48
Comments 51

CHAPTER VI

MAXIMAL OXYGEN UPTAKE IN NORWEGIAN SUBJECTS 53
Previous Investigations 53
53
School children 54
Student 54
Office-workers 54
Athletes 54
Results 55
Cross-sectional and longitudinal studies 55
Variation with sex and age 55
Maximal oxygen uptake in athletes 63
Comments 64

CHAPTER VII

GENERAL DISCUSSION 69

CHAPTER VIII

SUMMARY 84
REFERENCES 86
APPENDIX 95

PREFACE

The work on which the present monograph is based was carried out at the Institute of Work Physiology Oslo Norway; Department of Physiology Gymnastik och Idrottshögskolan Stockholm Sweden; and the Institute of Hygiene University of Oslo Oslo Norway

An investigation such as the one reported in this monograph is often the result of extensive and stimulating teamwork. It was also the case in this instance and there are several persons to whom I wish to express my sincere gratitude

To the heads of the institutes where the investigations were carried out Professor Kåre Rodahl, Professor Erik Hohwü Christensen and Professor Per-Olof Åstrand and to Professor Haakon Natvig, I express my thanks for financial support and laboratory facilities.

To Professor Kåre Rodahl I also wish to express my warmest gratitude for all the personal support and help I have received. He has been a source of continuous encouragement during the whole time that I have been writing this monograph

I have had the privilege of working with Associate Professor Bengt Saltin, whose support and advice during all phases of the work has been most inspiring

To Associate Professor Odd D Vellar Dr Björn Ekblom and Dr Milena Wachtlova I wish to express my warmest thanks for valuable and stimulating collaboration.

Excellent technical assistance was received from the staff of all three institutions. For their skilful help in collecting, analyzing and presenting the data, I especially wish to thank Miss Jorid Thrane, Mr Per-Ole Huser Miss Ragnhild Sidselrud and Mrs. Ingrid Liasen at the Institute of Work Physiology Oslo; Miss Gunilla Sparell, Mrs. Britt Marie Fjellner Ramning Mrs. Berit Pettersson-Sjöberg Miss Gunnel Nordberg and Mrs. Inger Hallbäck at the Department of Physiology Gymnastik och Idrottshögskolan, Stockholm; Miss Eva Rösholm Miss Anne-Sofie Brönn and Mrs. Aud Brevren at the Institute of Hygiene, Oslo

I gratefully acknowledge the help of Mrs. Joan Rodahl for correcting the language, checking the references and the final typing of the manuscript

I am also indebted to Dr Esther D R. Pruett, Dr Zdenek Vokac and cand.real Odd Vaage for stimulating discussion concerning the evaluation of the results.

My thanks are also due to Mr Sindre Varran for his valuable library assistance and to Mr Thor Endresen, Oslo and Mr Harry Hagelin, Stockholm for making or modifying necessary equipment for the performance of the various experiments.

I also wish to express my gratitude to Professor Kristian Lange Andersen who first introduced me into this field of physiology

Finally it is the author's pleasure to acknowledge gratefully the help willingness and cooperation of all of the 732 subjects who participated in these studies.

Oslo, February 1973 *Lars Hermansen*

CHAPTER I

GENERAL INTRODUCTION

The capacity to perform physical work has been a matter of primary interest for centuries. Clearly it must have been a subject of the utmost importance for our ancestors when survival depended upon their physical ability to provide food and adequate shelter for themselves and their families. The physical performance capacity of man is still a subject of considerable importance even for the people of today living in industrialized countries where the need for muscular work is claimed to be limited. The fact is, however, that in spite of the many laboursaving devices now available, a considerable amount of rather heavy physical work is still being done. It has been found, for instance, that a large number of the members of the Norwegian, as well as the Swedish Labour Unions regard their work as being physically heavy (Bolinder et al. 1969, Karlsen 1972).

The individual's physical performance capacity depends on a variety of factors including heredity, age, sex, training, state of health, motivation etc. The capacity to perform most ordinary daily activities, i.e. prolonged muscular work, depends on the individual's ability to transport oxygen from the air to the working cells. Under these conditions, the individual's maximal oxygen uptake is a useful indication of the over-all physical performance capacity.

The technique for measuring the over-all physical performance capacity in human subjects in terms of maximal oxygen uptake was developed by Hill and co-workers in 1924. In spite of the fact that physical performance capacity has been a subject of extensive studies for almost 50 years, and that the technique originally described by Hill et al. (1924) has in principal remained unchanged, there are still a number of theoretical and methodological problems to be solved.

The existing methodological problems may possibly be one of the factors explaining the large differences observed in the values for maximal oxygen uptake reported by different groups in various countries (Robinson 1938, Åstrand 1952, Åstrand 1960, Hettinger et al. 1961). However, more important are probably the differences in functional capacity due to hereditary and environmental factors, including daily levels of physical activity.

It is generally recognized that the capacity to transport oxygen to the working muscle cells is of primary importance for the individual's ability to perform muscular work. The oxygen transport in its turn depends on many interrelated functions, the relative importance of which needs to be assessed further. Of these, the functional capacity of the respiratory and the circulatory systems plays a major role.

A considerable reduction in the level of physical activity both on the job and

during leisure hours has been observed in many industrialized countries. This may conceivably affect the general level of physical performance capacity not only in adults but also in children. Knowledge of the general level of the physical performance capacity in the population is of considerable practical interest, not only as related to work and industry but also in connection with military defense, athletic standards and the maintenance of optimal health.

The purpose of the present investigation is to elucidate further some of the questions concerning the oxygen transport system in man More specifically the aim of the present investigation may be summarized as follows

1 to study the respiratory and circulatory responses to treadmill and bicycle exercise and to compare the results from these two most frequently employed testing devices.
2. to evaluate the effect of variations in the relative and total hemoglobin content of the blood on the maximal oxygen uptake.
3 to study the relationship between maximal oxygen uptake and capillary density (i.e. number of capillaries/mm^2) in subjects characterized by high as opposed to average maximal oxygen uptake
4 to describe the variation in maximal oxygen uptake in relation to sex, age and level of physical activity in samples of the Norwegian population.

In general, problem no 1 is dealt with in Study I and Study II. Problems no 2 and no 3 are considered in Study III and Study IV respectively Unpublished results are added in order to further elucidate questions raised in problems no 1 and 4

In the following the different problems are presented separately The description of the subjects and the specific methods and procedures are presented separately in each chapter However some of the methods and procedures are common in all studies, and are therefore described in the first part of the monograph

CHAPTER II

MATERIAL AND GENERAL METHODS

Subjects

Altogether 732 individuals between 10 and 68 years of age participated in the studies. Of these, 286 were females and 446 males. All female subjects were Norwegian, except one Swedish woman who took part in Study I The males consisted of 376 Norwegian, 66 Swedish and 4 American subjects. A further description of the subjects is given separately in each chapter of the present study and in the original papers (Studies I – IV) on which the present monograph is based

Methods

The oxygen uptake was determined by the Douglas bag method. The subjects breathed through a respiratory valve and the expired air was collected in Douglas bags. The respiratory valve had a dead space" of approximately 100 ml. The inner diameter of the valve, the stopcock and the tube of the Douglas bags was 30 mm The connecting tube between the stopcock and the valve was smooth and not corrugated with an inner diameter of 35 mm The total length of the tubes from the subject to the inside of the bag was approximately 50 cm The resistance of the air collecting system was measured during different constant flows. The results of these measurements are presented in Fig. 2–1 The volume of the expired air collected in the Douglas bags was measured in a wet spirometer (i.e. in all experiments performed in the laboratory) which could be read with an accuracy of 0.2 liter In the field studies (i.e. measurements of maximal oxygen uptake in the school children and in the office-workers) the volume of the expired air was measured in a dry gas meter The gas meter was calibrated against the wet spirometer before and after the experimental period. The dry gas meter could be read with an accuracy of 0.2 liter The gas analyses were performed on a Scholander apparatus (Scholander 1947) or on modified Haldane apparatus (Åstrand and Saltin 1961a) The accuracy of the gas analysis when using the Scholander apparatus was determined by making 10 analyses of the same test gas. The mean value and standard deviation were 6.23 and 0 02 vol.% and 15 83 and 0 03 vol.%, for the carbon dioxide and oxygen concentrations, respectively The accuracy of the oxygen measurements when using the Haldane gas analyzer is given by Åstrand and Saltin (1961a)

The heart rate was recorded by a conventional one channel electrocardiograph. Usually the heart rate was determined by counting the number of heart beats on 15 cm (i.e. 6 sec) of the recording paper However in a few experiments where

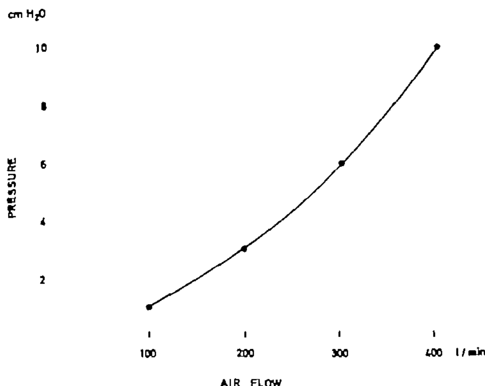


Fig. 2—1 Relationship between the valve pressure and air flow under steady state flow conditions.

maximal oxygen uptake was calculated from heart rate and work load (i.e. part of Study III) the heart rate was measured by palpation over the ictus cordis or the carotid artery using a special pulse-watch i.e. the time was taken for 30 heart beats.

The blood lactate concentration was determined either by the enzymatic method as described by Scholz et al. (1959) or by the Ström modification of the colometric method of Barker and Summerson (Ström 1949) Blood samples were taken from a prewarmed clean and dry finger tip The accuracy of the colometric method was tested by making 10 separate determinations in duplicate of the same blood sample The mean value and standard deviation were 11 07 and 0 49 mg/100 ml, respectively (Pruett 1971)

All experiments were performed at room temperature between 19 and 21 C, with a relative humidity of 45 to 60% and at sea level with a normal oxygen tension (i.e. 20 83 – 20 93 vol.%) The work experiments were performed on a motor-driven treadmill or on a bicycle ergometer The bicycle exercise was performed either on a mechanically braked bicycle ergometer as described by von Döbeln (1954) or on an electrically braked Krogh bicycle ergometer (Nielsen and Fridrichsen 1938)

Procedures

The submaximal work loads, if included in the protocol normally lasted for 6–10 min. Expired air for the oxygen uptake determinations was taken during the last 1–2 min of the exercise period. Heart rate was recorded every minute both before and during the period of gas collection The "steady state value obtained approximately at the 6th to the 7th min, i.e. immediately before the mouthpiece and the noseclip were placed on the subject, is given in the results.

The peak blood lactate concentration was determined by taking 2 to 3 blood samples during the last minute of the exercise and/or during the first 5 minutes after the exercise had stopped

All maximal work periods started with a 10 minute warm-up at a work load which represented approximately 50–70% of the individuals maximal oxygen uptake as calculated from the submaximal bicycle ergometer test (Åstrand and Ryhming 1954) As for the maximal work experiments on the bicycle ergometer the procedure suggested by Åstrand and Saltin (1961a) was used Maximal oxygen

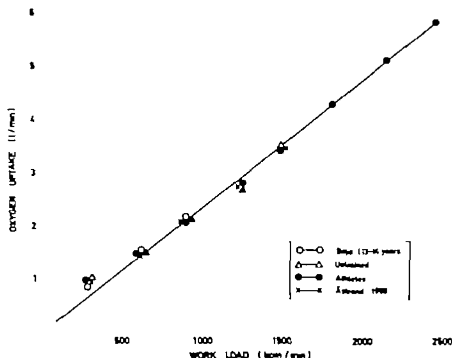


Fig. 2–2. Oxygen uptake (1/min) during submaximal bicycle exercise in untrained and well-trained adult subjects, and in boys. The work loads lasted for 10 min and the oxygen uptake measurements were performed between the 8th and th 10th min. Crosses denote results from Åstrand (1960) and open circles denot results from studies by Hermansen and Oseid (1971a)

uptake (1/min) was first predicted from the heart rate and submaximal work load on the bicycle ergometer according to the method of Åstrand and Ryhming (1954) With the aid of Fig 2–2 the work load was then chosen so as to be just high enough to reach the predicted maximal oxygen uptake. The work load for the next (second third or fourth) maximal exercise was increased by increments of 200 kpm/min. The pedal frequency during both submaximal and maximal work loads was set at 50 revolutions per min by a conventional metronome if not otherwise stated

On the treadmill a somewhat modified procedure originally described by Taylor et al. (1955) was used Also in this case the maximal oxygen uptake was first predicted from a submaximal test on the bicycle ergometer (Åstrand and Ryhming 1954) and expressed in ml/kg x min. With the aid of Fig 2–3 the speed for the first maximal run was chosen according to the same principles as described for the bicycle ergometer The speed for the next maximal run was increased by increments of 2 km/hr

The work time for the maximal exercise periods (both bicycle and treadmill) varied between 3 and 7 minutes. The work loads, both on the bicycle ergometer and the treadmill, were increased until the classical criterion with a "leveling off" was established i.e. that oxygen uptake increased less than 3% when the next maximal work load was increased by 200 kpm/min on the bicycle ergometer or 2 km/hr on the treadmill.

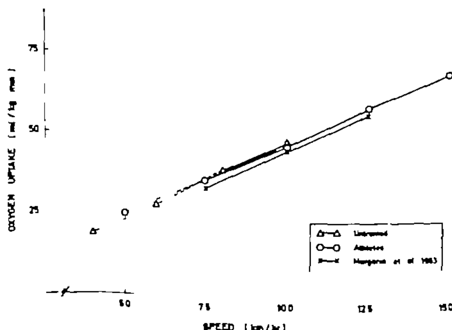


Fig. 2–3 Oxygen uptake (ml/kg x min) during submaximal treadmill exercise in untrained and well-trained subjects. Crosses denote results from Margaria et al. (1963)

The error of the maximal oxygen uptake measurements was calculated from 42 double determinations varying between 1 82 l/min and 5 57 l/min The mean value and the standard deviation were 3 94 and 0.08 l/min, and 3 90 and 0 08 l/min for the first and second determinations, respectively Furthermore, the reproducibility of the maximal oxygen uptake measurements was tested in one well-trained subject making 5 determinations within 13 days. The results of these experiments are given in Table 2–1

Statistics

The arithmetic mean (x) standard deviation (SD) and the standard error of the mean (SE) have been calculated according to standard statistical methods.

For the correlation analysis the formula for linear regression has been used the coefficient of correlation is obtained by

$$r = \frac{\Sigma (x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma ((x - \bar{x})^2 ((y - \bar{y})^2)}}$$

and the regression equation of y and x is:

$$Y = \bar{y} + b (x - x) = a + bX$$

where $\bar{x}$ and $\bar{y}$ denote the mean of the independent and the dependent variables, a is a constant and b is the regression coefficient obtained by

$$b = \frac{\Sigma (x - \bar{x})(y - \bar{y})}{\Sigma (x - x)^2}$$

The statistical significance was tested by means of the students t-test. The degrees of freedom were calculated from the number (n) of paired observations minus the number of 2 (i.e. 2n – 2) The conventional level of statistical significance, $p < 0\ 05$ was used.

The accuracy of a single determination of the different methods was calculated in most cases from 10 separate analyses of the same sample. The error of the methods could be read from the mean value and the standard deviation of these 10 separate analyses.

Table 2–1 Individual and mean values (with standard error of the mean and standard deviation) for oxygen ptake and related parameters obtained during 5 different maximal uptake determinations in the same subject within 13 days.

	Maximal values			
Examination	Oxygen uptake l/min	Heart rate beats/min	Pulmonary ventilation l/min	Blood lactate concentration mM
1st	5.57	181	244 0	10.4
2nd	5.54	181	207.3	10.3
3rd	5 49	180	215.3	10.7
4th	5.51	181	214.8	9.9
5th	5.46	181	216.0	9.8
Mean	5.51	181	219.5	10.2
SD	0.04	0.4	14.2	0.4
SE	0.02	0.2	7 1	0.2

CHAPTER III

RESPIRATORY AND CIRCULATORY RESPONSES TO DIFFERENT TYPES OF EXERCISE

Previous Investigations

In many types of muscular work there is a linear increase in oxygen uptake with increasing work load. This straight line relationship exists up to a critical level, beyond which the work load usually may be increased somewhat more, but the oxygen uptake eventually levels off or even declines (Taylor et al 1955 Mitchell et al. 1958a) This upper level of oxygen uptake is, according to Hill et al. (1924) defined as the individual's *maximal oxygen uptake*

It was shown by Christensen (1931) and later by others (Asmussen and Hemmingsen 1958 Åstrand and Saltin 1961b Stenberg 1966) that maximal work with the legs (i.e. large muscle groups) gives higher values for oxygen uptake than maximal work with the arms (i.e. small muscle groups) Taylor et al (1955) found a small but significant increase in the maximal oxygen uptake in one subject who was able to work an arm ergometer while running on the treadmill Furthermore, higher values for maximal oxygen uptake have been reported during skiing than during running or bicycling (Christensen and Högberg 1950 Åstrand 1952 Andersen et al. 1961). Åstrand and Saltin (1961b) measured oxygen uptake during different types of muscular work, including maximal work performed by arms, legs, and arms plus legs on a bicycle ergometer This investigation (Åstrand and Saltin 1961b) and later studies by Stenberg and co-workers (Stenberg 1966 Stenberg et al 1967) however did not confirm earlier observations (Christensen and Högberg 1950 Åstrand 1952 Taylor et al. 1955) showing that maximal work with arms plus legs gives higher values for oxygen uptake than those obtained during maximal exercise with legs "only" i.e. running or bicycling. Thus, according to the above cited studies it appears fairly well established that maximal exercise with the legs elicits higher values for oxygen uptake than does maximal work with the arms. However whether or not the oxygen uptake is higher during arm plus leg exercise than during leg exercise "only" is still an open question.

According to the studies cited above it appears that in order to load the respiratory and circulatory systems maximally large muscle groups must be engaged during the work. For this reason the bicycle ergometer and the treadmill are the two most frequently used laboratory testing devices for measurements of oxygen uptake and related parameters in human subjects. The testing procedures vary however from one laboratory to another and whether or not the two procedures give identical results is still an unanswered question. Åstrand (1952) for instance, found no significant difference in maximal oxygen uptake between maximal treadmill exercise (inclination 1 = 1.25%) and bicycling Later when Åstrand and Saltin (1961b) systematically compared the oxygen uptake during

different types of maximal exercise, a 5% higher oxygen uptake was found during maximal uphill running (inclination 3° = 5.25%) than during maximal bicycle exercise (50 rpm) The most pronounced difference in oxygen uptake comparing maximal work on the treadmill and on the bicycle was reported by Rowell and co-workers (Chase et al 1966 Rowell 1967) In 19 subjects they found on the average 0.60 l/min higher values on the treadmill than on the bicycle ergometer Leveling off was used as a criterion to establish the maximal oxygen uptake on the treadmill using the procedure described by Taylor et al (1955) On the bicycle, however they used the procedure described by Luft et al. (1963) with only one continuous exercise period. This fact may possibly explain the large difference observed in these studies. Glassford et al. (1965) using procedures very similar to those in the present investigation, reported mean values for maximal oxygen uptake which were 0.28 l/min higher during uphill running on the treadmill than during maximal bicycle exercise (50 rpm) Wyndham et al. (1966) have also demonstrated significantly higher (0.24 l/min) values for oxygen uptake during maximal treadmill exercise as compared with maximal bicycling In view of the somewhat contradictory results in the above cited studies, it is of importance to re-examine this question As degree and type of training as well as previous experience in maximal work tests may influence the results, different groups of subjects were studied.

Subjects

Altogether 4 female and 75 male subjects participated in the present investigation. Of these, 1 female and 57 males were included in Study I and 13 male subjects participated in Study II The remaining 3 female and 5 male subjects took part in the experiments in which oxygen uptake and related parameters were measured during uphill running and "ski-walking" on the treadmill. A further description of the subjects is given below

Study I included 1 female and 57 male subjects, all of whom were Swedish. The subjects were divided into 7 different groups according to age and level of physical activity (i.e. state of training)

Group A consisted of 8 endurance athletes (21–27 years old) Their average maximal oxygen uptake was 72 7 ml/kg x min.

Group B included 5 trained male students (mean age 22 0 years) Their average maximal oxygen uptake was 62.2 ml/kg x min

Group C: consisted of 6 untrained male students (mean age was 25.2 years) with an average maximal oxygen uptake of 49 1 ml/kg x min.

Group D consisted of 5 male subjects from the laboratory staff Their mean age was 30 0 years and their average maximal oxygen uptake was 61 3 ml/kg x min.

Group E: included 10 young athletes in cross-country running (orienteering) with an average age of 22 8 years. The mean value for maximal oxygen uptake in this group was 70 5 ml/kg x min.

Group F: consisted of 14 older athletes, still active in orienteering, age 45–68. The mean value for the maximal oxygen uptake in this group was 54 7 ml/kg x min.

Group G: Included 7 sedentary men (age 37–50 years) who were studied after 2 months of physical training (i.e. cross-country running). Their average maximal oxygen uptake was 45.9 ml/kg x min

Mean values for age, height and weight of the different groups are given in Study I. One female and 2 additional male subjects were used to study the effects of variations in the pedal frequencies on the oxygen uptake during maximal bicycle exercise.

Study II included 13 healthy male students who took part in the hemodynamic studies. Of these, 4 were American college students, aged 19–21 years. The mean value for maximal oxygen uptake in this group was 53.8 ml/kg x min. Only one of the subjects in this group was well-trained. In addition 9 well-trained Swedish subjects, with a mean maximal oxygen uptake of 67.4 ml/kg x min were included. Five of these 9 subjects competed regularly in endurance events. All subjects were familiar with the testing procedure. Individual values for age, height and weight are given in Study II.

In addition 3 female and 5 male well-trained subjects participated in the experiments with "ski-walking" and running on the treadmill. The mean values for age, height and weight of the 5 male subjects were 25.4 years, 180.4 cm and 73.2 kg, respectively. The corresponding mean values for the 3 female subjects were 25.3 years, 171.3 cm and 62.3 kg. The mean values for maximal oxygen uptake were 73.9 ml/kg x min in the male subjects and 56.9 ml/kg x min in the female subjects.

Methods and Procedures

Cardiac output was determined by the dye-dilution method using the same technique and procedures as described by Saltin et al. (1968) and Ekblom (1969). The error of the method was about 3–6% (Saltin et al. 1968, Ekblom 1969).

Arterial blood pressure was measured with the strain gauge system (Statham P23db or Elema, EMT 490) just before the cardiac output measurements were performed. The oxygen content of the blood was determined by the van Slyke technique or spectrophotometrically according to the method of Holmgren and Pernow (1959).

The cardiac output determinations were performed between the 6th and the 8th minute of exercise. During the maximal exercise the measurements were done during the last 2 min of exercise. Arterial blood pressure was measured just before the cardiac output determinations. Blood samples for measurements of oxygen content and blood lactate concentration were taken during the last minute of exercise. From the simultaneous (or almost simultaneous) measurements of cardiac output, blood pressure, oxygen uptake, heart rate and oxygen content of the arterial blood, the arteriovenous oxygen difference, peripheral resistance and the oxygen content of the mixed venous blood were calculated.

The "ski-walking" was performed on a large treadmill (3.3 meter long and 1.0 meter wide) with an inclination of 12 (21%). The speed of the treadmill was, in these experiments, varied between 60 and 160 m/min. The subjects were walking with slightly bent knees and were using the ski poles as in skiing (i.e. imitating the skiing movements), see Fig. 3–1. In order to obtain sufficient friction between the



Fig 3–1 "Ski-walking" with poles on the treadmill.

tips of the poles and the belt of the treadmill, and without damaging the belt, the metal spikes of the ski poles were removed and replaced by larger rubber knobs.

The measurements of oxygen uptake and other related parameters during "ski-walking" were performed in the same way as in the experiments when running on the treadmill.

Results

Oxygen uptake and related variables during treadmill and bicycle exercise

"Ski-walking" and running

The results of the determinations of the respiratory and circulatory responses to submaximal and maximal "ski-walking" and running on the treadmill are presented in Fig. 3–2 and in Table 3–1. During submaximal exercise in 2 subjects the heart rate was found to be somewhat higher during running than during "ski-walking"

with poles when compared at the same metabolic rate (i.e. oxygen uptake) The difference was almost the same (i.e approximately 10 beats/min) in both subjects, and at all work levels (Fig 3–2) The pulmonary ventilation however showed no consistent difference. There was a tendency towards higher values for the pulmonary ventilation during "ski-walking" than during running in the female subject (M.K.) However no difference was observed in the male subject (J.S) During maximal exercise in 8 subjects (Table 3–1) the mean values for oxygen uptake were found to be 4.82 l/min during ski-walking and 4 71 l/min during uphill running The difference, 0 11 l/min (2.3%) was statistically significant ($0.01 > p > 0.001$) The mean value for the pulmonary ventilation was also found to be higher during ski-walking compared with uphill running The difference 12.1 l/min was statistically significant ($p < 0.001$)

In spite of the above significant differences in the highest obtained values for oxygen uptake and pulmonary ventilation during "ski-walking and running there was no significant difference in the corresponding values for heart rate and blood lactate concentration (Table 3–1)

Treadmill and bicycle exercise

The results of the examination of the respiratory and circulatory responses to submaximal bicycle and treadmill exercise in well-trained and untrained subjects (i.e. Group A and C) are shown in Fig 2–2 and Fig 2–3 (Chapter II) and Fig 3–3 and Fig 3–4

The mean values for oxygen uptake during submaximal bicycle and treadmill exercise (Fig 2–2 and Fig 2–3 in Chapter II) were approximately the same as those reported by other investigators (Åstrand 1960 Margaria et al 1963) Heart rate, pulmonary ventilation and blood lactate concentration were found to be somewhat higher during bicycle exercise when compared with treadmill exercise at the same metabolic rates (Fig 3–3). For example heart rate was found to be on the average 8 10 12 and 14 beats/min higher on the bicycle than on the treadmill at oxygen uptakes of 1 0 1 5 2.0 and 3 0 l/min, respectively in the untrained subjects. The corresponding values in the well-trained subjects were 5 5 4 and 6 beats/min. The difference in heart rate between submaximal bicycle and treadmill exercise was found to be statistically significant ($p < 0.05$) This was also the case for the blood lactate concentration. However the difference in the pulmonary ventilation between submaximal bicycle and treadmill exercise was not found to be statistically significant ($p > 0.2$) The lower heart rate during submaximal exercise on the treadmill than on the bicycle at the same absolute oxygen uptake (i.e. l/min) gave a higher oxygen pulse during treadmill exercise. This was also the case, even when the difference in the maximal oxygen uptake values for the two types of work was taken into consideration (Fig 3–4)

The results of the determination of oxygen uptake, pulmonary ventilation, heart rate and blood lactate concentration during maximal exercise on the bicycle and on the treadmill in 55 male subjects are shown in Fig 3–5 and Table 1 in the Appendix.

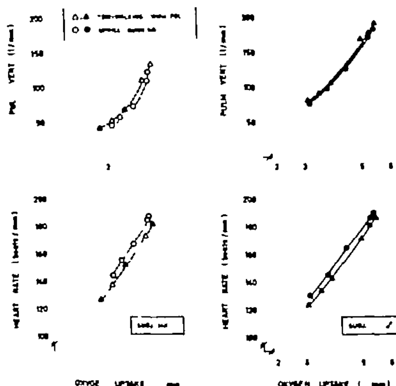


Fig. 3–2. Pulmonary ventilation (upper part) and heart rate (lower part) at different work loads during "ski-walking" and running on the treadmill in one female and one male subject.

Table 3–1. Mean values, ± standard error of the mean and standard deviation, for oxygen uptake, pulmonary ventilation, heart rate and blood lactate concentration during maximal ski-walking and maximal running (uphill, 3°) on the treadmill in 8 well-trained subjects.

		Maximal values			
Type of exercise	No. of subject	Oxygen uptake l/min	Pulmonary ventilation l/min	Heart rate beats/min	Blood lactate concentration mM
Uphill running	8	4.71 ± 0.36 0.96	137.8 ± 8.6 22.7	189.5 ± 2.6 7.0	11.1 ± 0.8 2.0
"ski-walking" with poles	8	4.82 ± 0.37 0.98	149.6 ± 10.2 27.0	187.6 ± 3.0 7.8	10.9 ± 0.9 2.4
Difference		0.01 > p > 0.001	p < 0.001	not signif.	not signif.

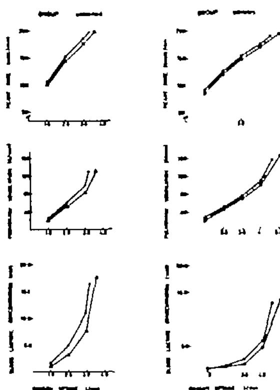


Fig. 3—3. Mean values for heart rate pulmonary ventilation and blood lactate concentration in relation to oxygen uptake during submaximal exercise on the bicycle ergometer (filled symbols) and on the treadmill (unfilled symbols) of untrained subjects (left panel) and athletes (right panel)

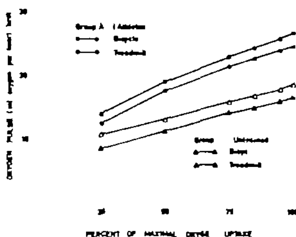
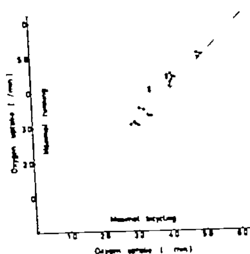
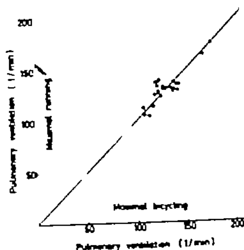
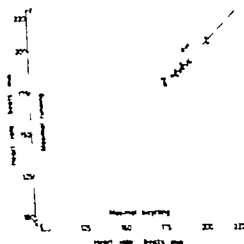


Fig 3—4. Mean values for oxygen pulse during submaximal and maximal bicycle and treadmill exercise in well trained (athletes) and untrained subjects.







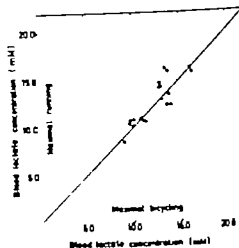


Fig 5-5 Individual values for oxygen uptake pulmonary ventilation, heart rate and blood lactate oncentration during maximal bicycle exercise (60 rpm) compared with orresponding values during maximal treadmill running (inclination 3)

As degree of training age as well as previous experience in maximal work tests might influence the results, the subjects were divided into different groups

The mean values for oxygen uptake during maximal treadmill exercise were found to be 0 41 0 34 0 37 and 0 31 l/min higher than the oxygen uptake during maximal bicycle exercise for groups A, B C and D respectively (Table 1 in the Appendix)

The differences were statistically significant ($p < 0\ 001$) Similar results were also obtained for the groups E-G although the differences were somewhat less pronounced The mean value for the total material (i.e 55 subjects) for the oxygen uptake during maximal treadmill exercise was found to be 4 18 l/min. The corresponding value obtained in the same subjects during maximal bicycle exercise was 3 90 l/min. The difference, 0 28 l/min (or 7 2%) was statistically significant ($p < 0\ 001$) The largest individual difference between the highest oxygen uptake values obtained during maximal treadmill and bicycle exercise, 0 61 l/min (or 14 0%) was seen in one of the cross-country runners (Group A) The largest difference observed in the untrained subjects (Group C) was somewhat lower (0 49 l/min) but expressed on a percentage basis, the difference was 18 7%. On the other hand, it should also be noted that 7 of the 55 subjects had higher values during maximal bicycle exercise than during maximal treadmill exercise. The largest individual difference in this direction was 1 9%. Thus, the individual values for the difference in oxygen uptake during maximal running compared with maximal bicycle exercise varied between + 18 7% to − 1 9%, with a mean difference of 7.2%.

In spite of the foregoing significant difference in the highest obtained values for oxygen uptake during maximal treadmill and bicycle exercise there was no significant difference in the corresponding mean values for pulmonary ventilation heart rate and blood lactate concentration (Fig 3−5)

Effect of variation in the pedal frequency (bicycling) and inclination (treadmill exercise)

Since a difference in oxygen uptake between maximal bicycle and treadmill exercise with the above test procedures was observed some supplementary experiments were undertaken in which the inclination of the treadmill and the pedal frequency on the bicycle were varied. The sequence for the bicycle and the treadmill tests was selected at random Table 3−2 summarizes the results from the experiments in which oxygen uptake during maximal running at no inclination is compared with the oxygen uptake during maximal running, uphill (i.e. 3−4.5°) The mean value for the maximal oxygen uptake during running at no inclination was 4 48 l/min. The corresponding value during uphill running was 4.68 l/min. The difference, 0.20 l/min, was statistically significant ($p < 0\ 001$) The mean value for pulmonary ventilation was also found to be lower during maximal running at no inclination when compared with uphill running The difference, 10 3 l/min (7%) was statistically significant ($p < 0.001$) In spite of the difference in oxygen uptake and pulmonary ventilation, no significant difference in the corresponding mean values for heart rate and blood lactate concentration could be observed

The results of the experiments concerning oxygen uptake during maximal

Table 3–.. Individual and mean values, & standard error f the mean and standard deviation, f xygen ptake pulmonary ventilation, heart rat and blood lactate concentration during maximal ru ing at 0 inclination and uphill ru ning (3.0–4.5) on the treadmill.

	Maximal values							
	Oxygen uptake l/min STPD		Pulmonary ventilation l/min BTPS		Heart rat beats/ml		Blood lactate concentration, mM	
Subjects	0	3.0–4.5	0	3.0–4.5	0	3 0–4 5	0	3.0–4.5
P–OA	4.28	4.37	124.8	131 7	193	186	9.8	15 1
BS	5.45	5.65	156.2	172.2	180	180	14 1	14 1
LH	4.28	4.47	161 7	164.9	196	194	11.2	12.6
LJ	3.88	4.09	112.4	125.2	193	190	5.4	11.6
RH	4 34	4 69	136.5	162.6	189	189	17 1	16 1
GL	4 65	4.81	161.3	157 9	197	202	14.8	17 0
Mean	4.48	4 68	140.5	150.8	191	190	12.1	14.3
SE	0. 4	0.24	8.6	8.3	.8	3.3	1.9	0.9
SD	0.53	0.54	19.3	18.6	6.2	7 4	4.2	1 9

bicycle exercise with 50 60 and 70 rpm are shown in Table 3–3 The mean value for oxygen uptake was found to increase from 3 96 l/min at 50 rpm to 4 06 l/min at 60 rpm. The difference of 0 1 l/min was found to be statistically significant ($p < 0\ 001$) However no significant increase was demonstrated if the pedal frequency was increased to 70 rpm The fact that there seems to be a peak value for oxygen uptake using pedal frequencies around 60–70 rpm was most markedly demonstrated in subject CS (Fig 3–6)

Hemodynamic response to submaximal and maximal bicycle and treadmill exercise

The results of the determinations of cardiac output and related variables at rest and during exercise on the bicycle and on the treadmill are shown in Fig. 3–7 to Fig 3–13 and Table 2 in the Appendix.

During submaximal exercise no significant difference in cardiac output was observed at a given oxygen uptake when the two work situations were compared (Fig 3–7) However the heart rate at a given oxygen uptake was found to be somewhat higher during submaximal bicycle exercise than during submaximal treadmill exercise This observation is in good agreement with the results of the experiments reported in Fig 3–4 Consequently the stroke volume was somewhat higher during submaximal treadmill exercise than during submaximal bicycle exercise However when the results for heart rate and stroke volume were related to the oxygen uptake expressed in percent of the individuals maximal oxygen uptakes obtained during bicycling and treadmill running no significant difference was observed (Fig 3–8) There was a considerable increase in the stroke volume

Table 3–3. Individual and mean values, & standard error of the mean and standard deviation, for oxygen uptake, pulmonary ventilation, heart rate and blood lactate concentration during maximal bicycle exercise with 50, 60 and 70 revolutions per minute.

Maximal values

Subjects	Oxygen uptake l/min STPD			Pulmonary ventilation l/min BTPS			Heart rate beats/min			Blood lactate concentration, mM		
	50 rpm	60 rpm	70 rpm	50 rpm	60 rpm	70 rpm	50 rpm	60 rpm	70 rpm	50 rpm	60 rpm	70 rpm
CS	5.45	5.60	5.54	172.9	179.7	192.7	198	198	198	17.1	18.8	18.1
LM	4.40	4.56	4.46	165.3	186.1	151.0	191	191	191	14.8	14.6	13.1
GL	4.09	4.23	4.16	141.2	146.1	154.1	194	195	193	13.1	17.2	14.2
LH	4.06	–	4.19	168.4	–	164.9	194	–	194	15.7	–	15.3
PL	2.91	3.01	3.00	104.1	114.3	116.1	188	186	187	12.1	11.8	10.9
EA	2.86	2.91	2.82	122.3	124.1	113.6	194	194	192	9.7	11.1	12.3
Mean	3.96	4.06	4.03	144.0	144.1	148.7	193	192	193	13.8	14.7	14.0
SE	0.44	0.56	0.45	12.0	13.0	13.5	1.8	2.2	1.6	1.2	1.5	1.1
SD	0.97	1.13	1.00	26.9	26.0	30.1	4.0	4.4	3.6	2.7	3.3	2.5

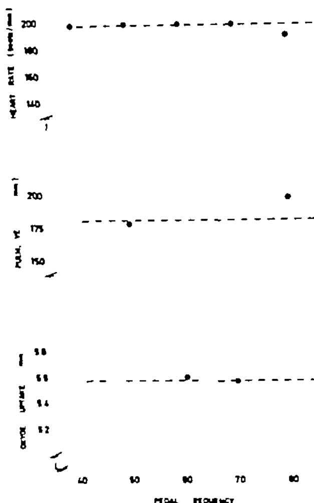


Fig. 3–6 Heart rate pulmonary ventilation and oxygen uptake during maximal bicycle exercise with different pedal frequencies (i.e. 40–80 rpm) in subjects CS. For comparison the value (dashed line) for maximal running uphill (3°) is also included. The work load was 2700 kpm/min in all experiments.

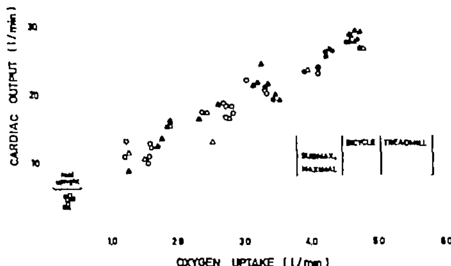


Fig. 3–7 Individual values for cardiac output in relation to oxygen uptake at rest and during submaximal and maximal bicycle and treadmill exercise.

from rest (sitting in an upright position on the bicycle ergometer) to light exercise (i.e. about 30% of the individual's maximal oxygen uptake). Beyond this work load there was a small further increase in the stroke volume with increasing work loads (Fig 3–8) Only on 4 occasions, 3 during treadmill exercise and 1 during bicycle exercise was the highest stroke volume observed during submaximal work loads. The values for the arteriovenous oxygen difference at rest and during exercise are shown in Fig 3–9 It will be seen that no consistent difference in the arteriovenous oxygen difference could be demonstrated between the two exercise forms (i.e. bicycle and treadmill) The values for the peripheral resistance, calculated from blood pressure and cardiac output, also showed no consistent difference between the bicycle and treadmill exercise (Fig 3–10)

A comparison of the hemodynamic data at maximal treadmill and bicycle exercise is shown in Fig 3–11 to Fig 3–13 and Table 2 in the Appendix.

In this study (Study II) the oxygen uptake during maximal bicycle exercise (50 rpm) was 4 31 l/min compared with 4 57 l/min during maximal treadmill exercise (inclination 3) The mean difference 0.26 l/min was statistically significant ($p < 0\ 001$) and almost identical to that observed in the 55 subjects in Study I

The mean values for cardiac output were found to be 27 5 l/min and 25 9 l/min during maximal treadmill and bicycle exercise, respectively The difference 1.6 l/min (or 6%) was statistically significant ($p < 0\ 001$) and the order of magnitude was approximately the same as the difference in the mean values for maximal oxygen uptake.

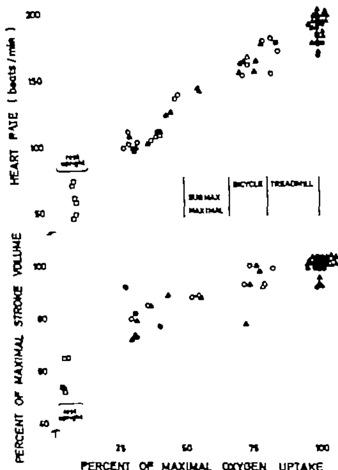


Fig. 3–8 Individual values for heart rate and stroke volume in relation to oxygen uptake expressed as percent of the individual's maximal oxygen uptake at rest and during submaximal and maximal bicycle and treadmill exercise.

The mean values for the heart rate during maximal exercise were 187 and 185 beats/min during treadmill running and bicycling, respectively. The difference was small but statistically significant ($p < 0.01$). Consequently, the stroke volume was higher during maximal treadmill exercise than during maximal exercise on the bicycle ergometer. The mean difference was approximately 5% ($p < 0.01$). Thus, the stroke volume was found to be higher both during submaximal and maximal treadmill exercise than during submaximal and maximal bicycle exercise.

On the other hand, no significant difference was observed in the arteriovenous oxygen difference between the two exercise forms (Fig. 3–9 and 3–11). However, both the mean arterial pressure and the peripheral resistance was found to be significantly lower during maximal treadmill than during maximal bicycle exercise

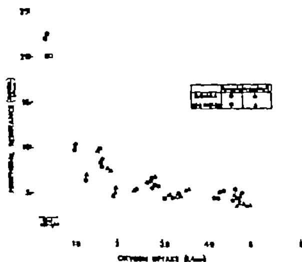


Fig 3–9 Individual values for arteriovenous oxygen difference in relation to oxygen uptake expressed as percent of the individual's maximal oxygen uptake during submaximal and maximal bicycle and treadmill exercise.

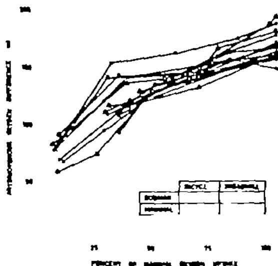


Fig. 3–10. Individual values for peripheral resistance in relation to oxygen uptake during submaximal and maximal bicycle and treadmill exercise.

Fig 3–11 Individual val es for cardiac output and heart rate (upper panel) and stroke olume and rteriovenous oxygen difference (lower panel) in relation to max mal oxygen uptake

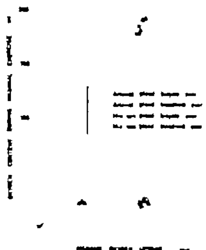


Fig. 3–12 Individual values for oxygen content in arterial blood (measured val es) and in mixed venous blood (calculated values) in relation to maximal oxygen uptake.

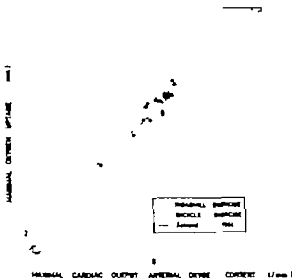


Fig. 3–13 Individual values for maximal oxygen uptake in relation to the total amount of oxygen distributed during exercise (i.e. maximal cardiac output x arterial oxygen content)

During maximal exercise with an oxygen saturation of 91–95%, the arterial oxygen content was 18 8 and 19 0 vol. % for treadmill and bicycle exercise respectively (Fig 3–12) As the maximal cardiac output was highest on the treadmill, the amount of arterially transported oxygen during maximal exercise was also higher on the treadmill compared with bicycle exercise (Fig 3–13) The mean values were 5 14 l/min and 4 93 l/min for the treadmill and bicycle exercise, respectively This difference, 0 21 l/min ($p < 0.01$) is approximately the same as the difference observed for the maximal oxygen uptake in the two types of work positions.

Comments

One of the major questions to be answered in the present investigation was whether or not the same values for maximal oxygen uptake can be obtained during maximal bicycle and treadmill exercise, using two standard procedures for obtaining the maximal values in the two types of work. By applying the leveling off criterion for *the estimation of maximal oxygen uptake the present investigation on 55* subjects in Study I and 13 subjects in Study II showed that oxygen uptake was 6–7% higher during maximal uphill running than during maximal bicycle exercise. It should however be noted that the individual variation was large, and the difference in oxygen uptake during maximal treadmill and bicycle exercise varied between plus 18 7% to minus 3 9%. It should also be emphasized that this variation was not related to the state of training of the subject previous experience in maximal exercise tests, or to previous training in bicycling

The hemodynamic studies during maximal bicycle and treadmill exercise showed that the higher oxygen uptake during treadmill exercise was explained by a higher cardiac output during running compared to bicycling This higher cardiac output was partly explained by a somewhat higher heart rate and partly by a higher stroke volume However in spite of the observed differences in oxygen uptake, heart rate and stroke volume, there were no significant *differences in* the values for pulmonary ventilation blood lactate concentration, arteriovenous oxygen difference or peripheral resistance between maximal treadmill exercise compared with maximal bicycle exercise

In summary it may be concluded that although there are some differences, the physiological responses to maximal treadmill and bicycle exercise are very similar The observed differences are relatively small and thus both treadmill and bicycle may be recommended On the other hand, the subjective feeling of fatigue or exhaustion during the maximal work necessary to obtain the maximal oxygen uptake is usually less when using the treadmill as compared to the bicycle ergometer Furthermore, on the basis of the results from the present investigation it may also be concluded that maximal oxygen uptake is only *maximal* for a specific type of exercise Consequently strict comparability of maximal oxygen uptake values requires strictly comparable and standardized conditions during the measurements. However if maximal oxygen uptake is measured during strictly standardized conditions, it is a highly reproducible parameter

CHAPTER IV

RELATIVE AND TOTAL HEMOGLOBIN CONTENT OF THE BLOOD AND MAXIMAL OXYGEN UPTAKE

Previous Investigations

The relationship between the hemoglobin content of the blood and maximal oxygen uptake (or physical work capacity) has been extensively studied by several investigators (Kjellberg et al. 1949 a,b Åstrand 1952, Sjöstrand 1953 Balke et al 1954 Robbe 1958 Sproule et al. 1960 Beutler et al 1960 Cotes et al 1969 Andersen and Barkve 1970 Ericsson 1970 and others) However the studies have yielded conflicting results.

Thus, Robbe (1958) found no influence of anemia (individual values for hemoglobin concentration as low as 8.2 g/100 ml) on the physical work capacity of pregnant woman. Cotes et al. (1969) made the same observation in nonpregnant anemic housewives (mean hemoglobin concentration 8 6 g/100 ml) Sproule et al (1960) on the other hand observed very low values for maximal oxygen uptake in 9 anemic patients in comparison with normal values for healthy subjects of comparable age. Several investigators (Kjellberg et al 1949a b Åstrand 1952 Sjöstrand 1953 Holmgren and Åstrand 1966) have emphasized that there is a close relationship between total hemoglobin content and maximal oxygen uptake (or physical work capacity PWC_{170}) Thus, Åstrand (1952) found that the correlation coefficient between maximal oxygen uptake and total hemoglobin content was 0 94 for the female and 0 97 for the male subjects, indicating that the total hemoglobin content might be an important determinant for maximal oxygen uptake.

A reduction in the total amount of hemoglobin caused by bleeding has been shown to cause a lowering of the physical work capacity (Karpovich and Millman 1942 Balke et al. 1954) Rowell et al (1964) on the other hand showed that a reduction in hemoglobin concentration of 14% due to repeated phlebotomies during an eight-day period gave almost no reduction in maximal oxygen uptake.

In spite of the fact that several investigators have examined the relationship between maximal oxygen uptake and hemoglobin concentration apparently no one has studied the effect of altered hemoglobin content brought about by increasing the dietary iron intake, in a longitudinal type of study

The aim of the present investigation was to study the effect of altered hemoglobin content on the maximal oxygen uptake both in cross-sectional and longitudinal studies.

Subjects

Altogether 284 subjects participated in the study Of these 183 were school children, 97 were physical education students and 4 were patients with iron deficiency anemia All the 284 subjects participated in Study III which also included a supplementary group of 8 female physical education students. However as the results obtained in these subjects did not give any new information they were not included in this monograph

School children

The school children material which included 95 boys and 88 girls was obtained from the pupils of two elementary schools (i.e. Kjøllefjord and Bjørnevatn) in Finnmark. Pertinent data describing the subjects are given in Study III

Physical education students

47 female and 50 male students participated in the study The mean age was 24 5 years for the men and 22 5 years for the women The mean values for maximal oxygen uptake (calculated from heart rate and work load during submaximal bicycle exercise) and hemoglobin concentration for the male students at the start of the experiment were 47 5 ml/kg x min and 15 1 g/100 ml, respectively The corresponding values for the female students were 40 8 ml/kg x min and 12.91 g/100 ml Further information about the subjects is given in Study III

Selected cases of iron deficiency anemia

During the period of March – December 1969 four cases of iron deficiency anemia were found suitable for examination of the maximal oxygen uptake (i.e. maximal running on the treadmill) Pertinent data describing these patients are given in Table 4–1

Methods and Procedures

The determination of the hemoglobin concentration in the present study was performed by the cyanmethemoglobin method (Larsen 1966) The accuracy of the method was tested by taking 10 separate determinations of the same blood sample The mean value and standard deviation of the 10 analyses were 13 3 and 0 3 g/100 ml

The total amount of hemoglobin was calculated from the determinations of hemoglobin concentration and the blood volume The blood volume was calculated from measurements of the hematocrit and the plasma volume. The hematocrit was measured in heparinized capillary tubes after centrifugation in an hematocrit centrifuge. The accuracy of the hematocrit measurements was tested in the same way as for the hemoglobin concentration. The mean value and the standard deviation were 43 1 and 0 9%. The plasma volume was measured by the radioiodine plasma protein method of Williams and Fine (1961) The error of the method was not determined

The experiments involving school children were performed between October 1968 and April 1969 The first examination in October 1968 included 72 school

Table 4–1 Pertinent data describing the anemic subjects.

Subjects	sex	age	height	weight	Hb concentration (g/100 ml) at start	Follow up period (weeks)	Treatment
To.He. (blood donor)	male	45	182	86	14.2	13	Iron
T.H. (priv.pract. patient)	female	55	171	58.2	10.9	7	Iron
R.L. (blood donor)	female	35	170	57 9	11 0	20	Iron
S.H. (blood donor)	female	18	165	69.5	11 4	26	placebo 4 week iron 22 weeks

children (i.e. 39 boys and 33 girls) The second (final) examination was performed on the same subjects in April 1969 However in this final examination an additional material of 56 boys and 55 girls was included Thus, the final examination included altogether 183 subjects, i.e. 95 boys and 88 girls. The hemoglobin concentration was measured on the same day and prior to the maximal oxygen uptake determinations. Maximal oxygen uptake was determined by the Douglas bag method in all school children both at the first and the second examination. No iron medication was given to the school children.

The experiments on the physical education students were performed between August 1967 and May 1968 According to the results from the measurements of hemoglobin concentration at the start of the experiment and to the criteria of normal hemoglobin values in Norwegian men and women (Natvig et al. 1967) the subjects were divided into two groups

Group A with normal hemoglobin values
Group B: with subnormal hemoglobin values

By paired sampling technique all subjects in Group A (i.e. with normal Hb values) were divided into two subgroups

Group 1 the iron therapy group
Group 2: the placebo group

All subjects in Group A and Group B were given iron or placebo tablets. Iron was given in the form of ferrofumerate. One iron tablet was equivalent to approximately 60 mg of bivalent iron. The iron tablets were of identical shape and size and labelled "ferrofumerate X" and ferrofumerate Y" respectively Each person received the tablets packed in boxes of 100 and was instructed to take one tablet once a day regardless of the blood values at the start of the experiment The tablets were taken in the evening Re-examination of the hemoglobin concentration and

maximal oxygen uptake was performed with approximately eight weeks intervals, the last one in May 1968 Including the first examination six examinations were made althogether The actual consumption of iron was recorded at the end of the experimental period (i.e. after 40 weeks) Maximal oxygen uptake was calculated from heart rate and work load during submaximal bicycle exercise according to the method of Åstrand and Ryhming (1954) in all female and male students. However maximal oxygen uptake was also measured using the Douglas bag method, in a sample of 20 male and 21 female students at the first examination (i.e. Aug 1968) and in 16 male and 15 female students at the sixth examination (i.e. May 1969) Total hemoglobin content was measured in 20 students at the first examination (Aug 1968) and in 22 students at the sixth examination (May 1969)

The anemic patients were studied for periods of 7 to 26 weeks. Maximal oxygen uptake and hemoglobin concentration were measured at several re-examinations in the same patient. The interval between the re-examinations was usually about one week but varied between 3 and 44 days. All determinations of maximal oxygen uptake in the anemic subjects were performed using the Douglas bag method. None of the subjects donated blood during the period of investigation. Iron tablets were administered to 3 of the patients from the start of the experiments. The dose was 2–3 tablets a day The 4th subject received placebo tablets during the first 4 weeks. From then on however the placebo tablets were replaced by iron tablets. Otherwise, the scheme of administration was similar to that described for the students.

Results

Cross-sectional analysis

The results of the determination of hemoglobin concentration and maximal oxygen uptake in the various age groups of the school children are given in Fig. 4–1 The mean values for the hemoglobin concentration were fairly well in accordance with the proposed normal values of Natvig et al. (1967) for school children of comparable age In some groups, however the mean values were below the previously proposed standards. The values for maximal oxygen uptake were, on the average slightly higher than the values given by Åstrand (1952) for school children in Stockholm of comparable age.

It will be seen from Fig 4–1 that the mean values for maximal oxygen uptake in the boys increased almost linearly with increasing age from the age of 10 to 14 years From then on, the maximal oxygen uptake continued to increase although at a somewhat slower rate The mean values for the hemoglobin concentration on the other hand showed no systematic changes in the age groups 10–13 years. However from the age of 13 to 16 years, the hemoglobin concentration (mean values) increased from 13 2 to 14 5 g/100 ml. In the girls, the maximal oxygen uptake showed a marked increase from 10 to 13 years. From then on, no change in maximal oxygen uptake in relation to increasing age was observed The mean values for the hemoglobin concentration in the schoolgirls showed no systematic changes

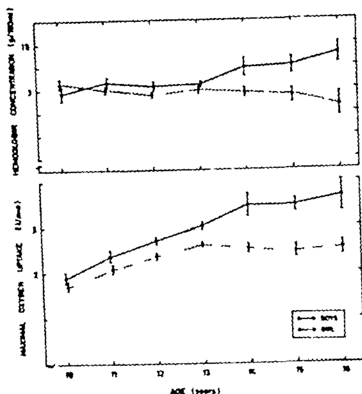


Fig. 4–1 Mean values (± SE) for hemoglobin concentration and maximal oxygen uptake in various age groups of school children.

with increasing age. However the hemoglobin concentration (mean values) were somewhat higher for the 10 year-old girls and somewhat lower for the 16 year-old girls than for the other age groups examined

The correlation between hemoglobin concentration and maximal oxygen uptake was tested in two sets of values. The results from the first examinations on school children and students were pooled and designated the *1st examination* although the investigations in the two groups were undertaken at different calendar times In the same way the results obtained in the final examinations n both school children and students were pooled and designated the *2nd examination*. The results obtained in each of the examinations have been analyzed separately Thus, the correlation coefficients (see Table 3 in the Appendix) refer to one set of values in each individual. It should also be emphasized that the correlation coefficients given in Table 3 in the Appendix are based on the *measured* (i.e. Douglas bag method) values for maximal oxygen uptake only both in the student and the school children material.

There was a statistically sign ficant positive correlation between the hemoglobin concentration and the maximal oxygen uptake (l/min) both at the 1st and the 2nd

examination. However when the data for maximal oxygen uptake were expressed in ml/kg x min no definite statistical relationship could be demonstrated At the first examination where 41 students and 68 school children were studied, the correlation coefficient was 0 07 i.e not significant However at the second examination a statistically significant relationship was found ($p < 0.001$) It should be noted however that the number of school children investigated had been markedly increased from the first to the second examination (i.e from 68 to 177)

In order to make further analysis of the relationship between hemoglobin concentration and maximal oxygen uptake the correlation coefficients in the various subgroups (male and female students, schoolboys and schoolgirls) have also been calculated With two exceptions, none of the subgroup correlation coefficients were statistically significant The general finding is further illustrated in Fig 4-2 where corresponding values for hemoglobin concentration and maximal oxygen uptake (l/min) for the students and school children at the first examination are given (i.e. total material) It is evident that, although the scatter of the individual values around the calculated regression line is considerable the correlation between the two parameters is positive and significant ($r = + 0.67$) However within the four subgroups, which are encircled in the figure there was no definite correlation between the two parameters. Although there is a wide scatter and a great deal of

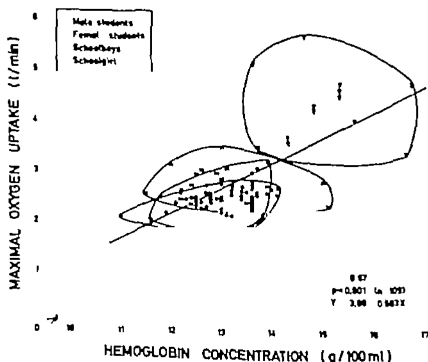


Fig 4-2 Corresponding values for hemoglobin concentration and maximal oxygen uptake. The values in each of the subgroups (i.e schoolgirls, schoolboys, female students and mal students) are encircled

overlapping in the individual values in the various subgroups, there is a tendency to a concomitant increase in both hemoglobin concentration and maximal oxygen uptake from subgroup to subgroup in the following order schoolgirls, schoolboys, female students and male students. A similar covariation between the changes in hemoglobin concentration and maximal oxygen uptake may also explain the finding of a positive correlation in the school children (i.e. schoolgirls, first examination and schoolboys, second examination) In both cases, small individuals are compared with others who were nearly full-grown

The relationship between total amount of hemoglobin and maximal oxygen uptake in 20 students at the first examination (i.e. Aug 1968) and 22 students at the second examination (i.e. May 1969) was found to be statistically significant in both examinations, ($p < 0.001$) However the close correlation which was observed in the total student material (i.e. both female and male students could not be confirmed when each sex was examined separately The same lack of relationship was found when the correlation coefficients were based on the maximal oxygen uptake values in ml/kg x min (i.e. after correction for differences in body weight) However the number of subjects in each group was small

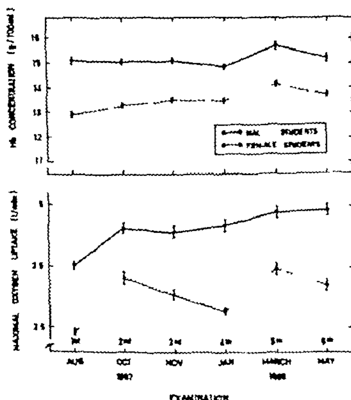


Fig. 4–3 Mean values (± SE) for hemogl bin concentration and maximal oxygen uptake in female and male students during the course of the experimental period

Longitudinal analysis

The results of hemoglobin concentration and maximal oxygen uptake from the six examinations in the students are represented in Fig 4–3

Although there were some fluctuations in the values from examination to examination there was a pronounced increase in maximal oxygen uptake during the 9-month examination period both in the male and female students. The mean increase in maximal oxygen uptake from the first to the final examination was approximately 12 ml/kg x min in both the male and female students, representing an increase of 25 and 31% respectively It should however be noted that these values for maximal oxygen uptake were not measured directly but calculated from heart rate (and work load) during submaximal exercise on the bicycle

The hemoglobin concentration also showed a small increase (i.e. 7% over the initial value) during the same period in the female students. However the mean values for hemoglobin concentration in the male students remained almost unchanged during the whole examination period (i.e 9 months)

The results of the studies concerning the effect of iron supplements on the hemoglobin concentration and maximal oxygen uptake are presented in Fig 4–4 It will be noted that the subgroups within each sex were similar both with regard to the hemoglobin concentration (with exception of the subnormal group) and maximal oxygen uptake at the start of the experimental period

The same increase in maximal oxygen uptake as shown in all male and all female subjects (Fig 4–3) was also found in the different subgroups. Although there were some fluctuations in the values, no systematic difference between the different subgroups could be demonstrated The hemoglobin concentration increased in all female subjects, regardless of whether iron or placebo tablets were consumed The largest increase was observed in the subnormal group A similar increase however was not found in the male subgroups. Furthermore from the results in Fig 4–4 it is evident that the maximal oxygen uptake may increase and the hemoglobin concentration decrease or vice versa, from one examination to another

The assessment of the actual consumption of tablets (iron and placebo) at the end of the experiment revealed that the subjects had followed the instructions to take one tablet a day during the 40-week experimental period The average consumption per individual was 270 tablets, and thus in good agreement with the proposed consumption according to the plan of the investigation i.e approximately 280 tablets.

Selected cases of iron deficiency anemia

The results of the studies concerning the relationship between hemoglobin concentration and maximal oxygen uptake in 4 selected cases of iron deficiency anemia are given in Fig. 4–5 All subjects were given iron tablets during the whole experimental period with the exception of subject S H who received placebo tablets for 4 weeks and from then on iron treatment was given according to the same scheme as for the 3 other subjects. In two subjects (i.e To.He. and T H) there was a pronounced increase in the hemoglobin concentration. Maximal oxygen uptake also showed a slight increase, but only from the first to the second

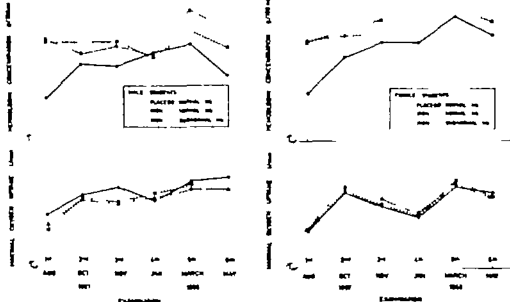


Fig. 4—4. Mean values for hemoglobin concentration and maximal oxygen uptake in the 3 subgroups (i.e. the iron therapy group the placebo group and the group with subnormal hemoglobin values at the start of the experiment) in the male students (left panel) and in the female students (right panel) during the course of the experimental period.

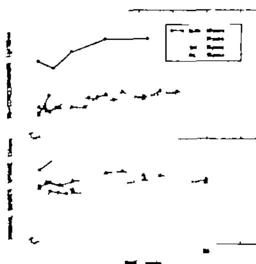


Fig. 4—5 Individual values for hemoglobin concentration and maximal oxygen uptake in 4 anemic patients during the course of th experimental period. All subjects were given iron tablets (i.e. 1°0—180 mg per day) during the whole experimental period. The only exception was S.H. who received placebo tablets for 4 weeks. From then on iron medication was given according to the same schem as for the 3 other subjects.

examination From then on, the values for maximal oxygen uptake remained almost unchanged For the two other subjects (i.e. S H and R L.) both hemoglobin concentration and maximal oxygen uptake varied during the course of the 20–24-week examination period However no definite relationship seemed to exist between the variations of the two parameters examined.

Comments

From the results of the present investigation it is evident that the values for both relative and total hemoglobin content vary considerably from one individual to another and also from time to time in the same individual It should also be emphasized that the variations in the hemoglobin concentration were approximately the same in the placebo and the iron therapy group A crucial question is of course whether or not the students followed the instructions for taking the iron tablets given by the investigators. However the assessment of the actual consumption of tablets (iron and placebo at the end of the experiment) revealed that the subjects had followed the instructions fairly well The average consumption per individual during the experimental period was about 270 tablets, and this was in good agreement with the theoretical consumption according to the plan of the experiment (i e. approximately 280 tablets)

A corresponding large variation was also observed in the values for maximal oxygen uptake. The most pronounced variation was observed in the female subjects who showed a marked increase from the first to the second examination (Fig 4–4) During this period the students were subjected to regular outdoor training (cross-country running and field exercises) However during the period from October to January the activity program was changed to indoor activities (i.e. gymnastics) During this time a pronounced fall in maximal oxygen uptake was observed The 5th examination was performed in March immediately after a period with outdoor activities (i.e. skiing) The variations in the male subjects were smaller although they followed approximately the same pattern as for the female subjects.

In the student material most of the values for maximal oxygen uptake were estimated by the indirect Åstrand Ryhming method (Åstrand and Ryhming 1954). Although this method does not provide the same degree of accuracy as the direct Douglas bag method, it is reasonable to believe that it may give valuable information about the longitudinal changes as each individual serves as his own control Furthermore the pronounced increase in maximal oxygen uptake found in the female students from the first to the second examination was confirmed in a group of 8 students studied with Douglas bag method The mean increase in maximal oxygen uptake of the 47 female physical education students was 0 90 l/min from the first to the second examination. The corresponding increase in the 8 female students studied with the Douglas bag method was 0 57 l/min Thus, it seems unlikely that methodological errors may account for more than a fraction of the observed total variations in the maximal oxygen uptake during the 9-month experimental period It should also be emphasized that with regard to the correlation analysis, only values for maximal oxygen uptake determined with the Douglas bag method were used

CHAPTER V

CAPILLARY DENSITY OF SKELETAL MUSCLE AND MAXIMAL OXYGEN UPTAKE

Previous Investigations

The main function of the capillaries is to distribute oxygen, nutritive materials and hormones to the various cells of the body and to collect end products of the metabolism for further transportation to the excretory organs.

The capillary density is essential for all of these functions. For instance analyses of the oxygen transport in peripheral tissues require information about the differences in the pO_2 of the various compartments between the erythrocytes and the mitochondria. Direct measurements of the pO_2 gradients have been difficult to obtain, and several indirect methods have been developed The classical approach to the problem has been to calculate the pO_2 gradients from the diffusion coefficient for oxygen in the tissue, the metabolic rate and the dimensions of the capillary bed (Krogh 1929) However these calculations are only as good as the data on which they are based, the number of capillaries per mm^2 being one of the most important parameters. From the studies of Petrén et al 1937 Vannotti and Pfister 1933 Vannotti and Magiday 1934 it has been assumed that the increase in physical performance after training was associated with an increase in the number of capillaries per mm^2 in the trained muscle. However results for measurements of the capillary density in human skeletal muscle in connection with bed rest and training (Saltin et al. 1968) do not support this view Thus, morphological studies in animals and human subjects concerning the capillary density in well-trained and untrained muscle have yielded conflicting results.

Subjects

Altogether 23 male subjects, 13 athletes and 10 untrained students participated in the study Their age varied between 22–32 years. Of these, 7 athletes and 8 students were included in Study IV In addition 6 athletes and 2 students were studied. However due to technical difficulties the results of these subjects (i.e. 6 athletes and 2 students) were not as complete as the material presented in Study IV In the primary material (7 athletes and 8 students) biopsy samples from the lateral portion of the quadriceps femoris muscle were obtained both before and after maximal exercise In the additional material samples were obtained either before or after the work. All athletes had been engaged in hard physical training and competition for at least 5 years. All of them participated in endurance events, i.e. marathon running orienteering and bicycling (road racing) They were all successful athletes of high national standard Their average maximal oxygen uptake

was 71.3 ml/kg x min (n = 13). The student material included a total of 10 subjects. None of the students had been engaged in any regular physical training during the last 3 years. Their average maximal oxygen uptake was 52.0 ml/kg x min (n = 10).

Methods and Procedures

The capillary density was determined in small biopsy specimens (10–20 mg wet weight) from the lateral portion of the quadriceps muscle. The biopsies were performed according to the method of Bergström (1962). The tissue specimens were immediately (i.e. within 5 – 10 sec after the biopsy was finished) immersed in 10% formalin. After a fixation period of one month or more the specimens were treated according to standard histological procedures (i.e. immersed in alcohol and embedded in paraffin). After sectioning the periodic-acid Schiff (PAS) reaction according to the method of Hecht (1958) was used. The number of capillaries and muscle fibres were counted in a microscope with a vertical camera. Twenty fields were calculated from each specimen using 18–20 different sections. The size of the field used for all calculations was 40 000 μ^2. The capillaries and muscle fibres were only calculated in cross-section. From these two measurements two other parameters were calculated, i.e. the capillary/fibre ratio and the diffusion distance according to the method described by Krogh (1919). The diffusion distance is defined as the average half distance between two capillaries on the cross-section.

Two biopsies were performed in each subject. The first biopsy was taken after the subject had been resting in the laboratory for 20–30 min. The last one was taken immediately (i.e. within 5–10 sec) after the cessation of 4–6 min maximal exercise on the treadmill. The maximal oxygen uptake was determined on separate days.

Results

Individual values for the number of capillaries per mm^2 and the number of muscle fibres per mm^2 in small muscle specimens obtained in the lateral portion of the quadriceps femoris muscle and maximal oxygen uptake in athletes and students are given in Fig. 5–1 and Fig. 5–2. It appears that (Fig. 5–1) although the scatter of the individual values is quite large the number of capillaries per mm^2 in the untrained students and in the well-trained athletes were approximately the same. The mean values were 579 and 587 capillaries per mm^2 in the students (n = 10) and the athletes (n = 13) respectively. Statistical analysis revealed no significant difference. However, as will be seen from Fig. 5–2 the number of muscle fibres per mm^2 was smaller in well-trained than in untrained subjects. Thus, the average size of the muscle fibres in the well-trained subjects was approximately 30% larger than in the untrained muscle. The difference was statistically significant ($p < 0.001$).

Since the number of muscle fibres per mm^2 was lower in the well-trained than in the untrained group and the number of capillaries per mm^2 was the same in both groups, the capillary/fibre ratio was found to be significantly higher ($p < 0.05$) in the well-trained group. On the average, each muscle fibre from the well-trained

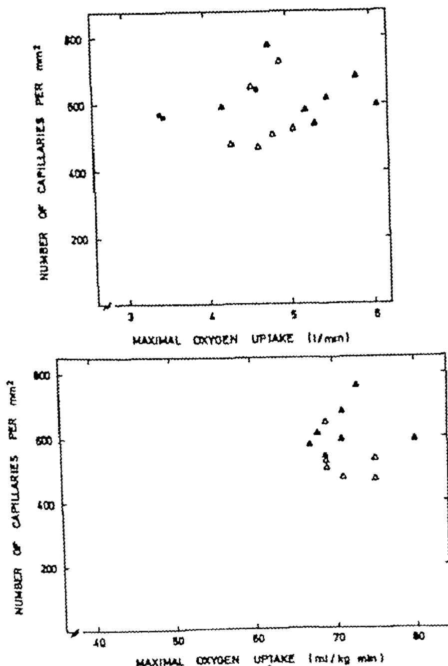


Fig. 5–1 The number of capillaries per mm^3 in small muscl specimens obtained from the lateral portion of th quadriceps femoris muscle of athletes (Δ,▲) and untrained students (o ●) in relation to the individuals maximal oxygen uptake expressed as l/min (upper panel) and ml/kg x min (lower panel) The filled symbols denote mean values from samples obtained before and after maximal exercise. The unfilled circles and triangles denote single values obtained from samples taken before or after maximal exercise

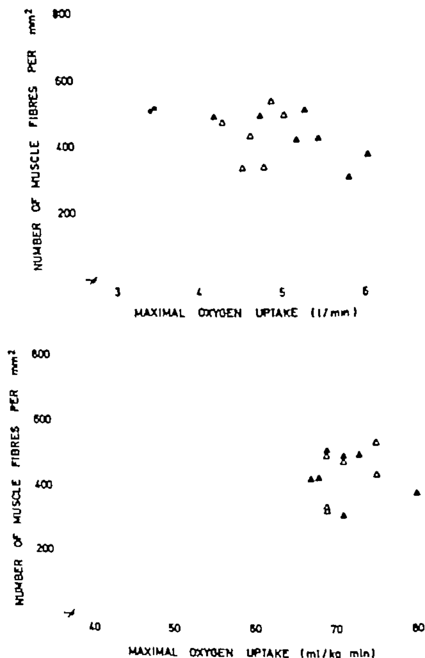


Fig. 5–2. The number of muscle fibres per mm² in small muscle specimens obtained from the lateral portion of the quadriceps femoris muscle of athletes (Δ,▲) and untrained students (o ●) in relation to the individuals maximal oxygen uptake expressed as l/min (upper panel) and in ml/kg x min (lower panel). For explanation of the symbols see Fig. 5–1

muscle was supplied with 1 5 capillaries, whereas in the untrained muscle the ratio capillaries to fibres was approximately 1

However when the diffusion distance i.e average half distance between the capillaries, was calculated according to the method of Krogh (1929) no statistically significant difference between the well-trained and untrained muscle was observed

Comments

The capillary density of heart and skeletal muscle has been studied by several investigators, mainly by means of infusion techniques or by counting of erythrocyte-packed capillaries (for references see Landis and Pappenheimer 1963 Hammersen 1968) These methods have however been subject to criticism (Hort 1968) since not all capillaries are necessarily filled with ink or packed with red blood cells. However the method used in the present investigation i.e. the PAS staining reaction is considered to be specific to the polysaccharides in the basement membrane of the capillaries. Thus, with this method it should be possible to count all capillaries, whether packed with erythrocytes or not or whether the capillaries are open or closed

In order to make a further evaluation of the method for estimating capillary density in the present investigation, muscle samples were obtained at rest and immediately after maximal exercise in the same subjects. According to Krogh (1929) the number of open capillaries in the skeletal muscle is markedly increased during exercise. Although there is a rapid fall in the muscle blood flow after cessation of work (Grimby et al. 1967) the blood flow during the first 5—10 sec of the recovery period is still very high compared with resting values. Normally all biopsies were completed during this time period. Thus, when the first biopsy was performed only a few capillaries were open whereas when the second biopsy was taken the number of open capillaries was much higher The results obtained both in the well-trained and untrained subjects, showed that there was no statistically significant difference between the values for capillary density in samples taken before or immediately after the maximal exercise. Consequently the method used in the present investigation may be considered to give a fairly reliable estimation of the number of capillaries per mm^2 in the muscle samples of the two groups of subjects in the present investigation.

The number of capillaries per mm^2 in the present study was found to vary between approximately 400—800 (average about 600 capillaries per mm^2) both in the well-trained and in the untrained subjects. Cap llary counts based on an infusion technique or counting of erythrocyte-packed capillaries led to estimates in the range of 200—600 capillaries per mm^2 in resting skeletal muscle and to 600—5000 capillaries per mm^2 for working skeletal muscle (Landis and Pappenheimer 1963) On the other hand values of 150—200 cap llaries per mm^2 are reported in other studies using electrone microscop c methods or by counting of capillaries in frozen sections of skeletal muscle (Landis and Pappenheimer 1963 Saltin et al. 1968)

This large variation in the number of capillaries per mm^2 may at least partly be explained by the fact that almost all ordinary histological techniques produce some shrinkage of the muscle tissue. Consequently direct comparison of the absolute values for capillary density from one study to another may be of limited value. However in the present study the same fixation and staining procedures were used for all tissue specimens, both from the well trained and untrained muscles. Therefore the shrinkage is considered to be approximately the same in both groups of subjects.

CHAPTER VI

MAXIMAL OXYGEN UPTAKE IN NORWEGIAN SUBJECTS

Previous Investigations

Maximal oxygen uptake differs greatly from one individual to another (Robinson 1938) The value is dependent upon the body wieght or more accurately the muscle mass of the subjects (Buskirk and Taylor 1957) Maximal oxygen uptake is also *dependent upon the individual s level of physical activity* (Åstrand 1952 Hermansen and Andersen 1965 Saltin and Åstrand 1967) Furthermore, maximal oxygen uptake first increases and later decreases with increasing age (Åstrand 1952 Åstrand 1960 Andersen et al 1961) and is lower in female than in male subjects (Åstrand 1952, Åstrand 1960) Most studies describing the variations in maximal oxygen uptake with sex and age have been performed as cross-sectional studies. Apparently no one has examined these variations in a longitudinal type of study Consequently it is not known whether or not the results obtained in cross-sectional and longitudinal studies are comparable

The variation in maximal oxygen uptake with sex, age and level of physical activity are rather well established in some countries, such as Sweden (Åstrand 1952, Åstrand 1960 Saltin and Åstrand 1967) and U.S.A. (Robinson 1938 Mitchell et al. 1958a, Hettinger et al 1961 Rodahl et al 1961 Knuttgen 1967) Although some reports on maximal oxygen uptake in samples of the Norwegian population are already published (Andersen et al. 1961 Hermansen and Andersen 1965 Andersen and Hermansen 1965) it is of interest to give a more complete picture of the variations with sex, age and level of physical activity Both cross-sectional and longitudinal studies are included

Subjects

Altogether 593 subjects are included in this study The material consists of school children untrained and trained students, office-workers and different groups of well-trained athletes.

The results of the school children are part of a larger study (to be published) dealing with the problems of the relationship between maximal oxygen uptake and the body dimensions. However although this question may also be regarded as important when describing the changes in maximal oxygen uptake with sex, age and level of physical activity it is not included in the present publication

School children

The school children material consisted of pupils from 3 different elementary schools in the county of Finnmark i.e. the schools in Kjøllefjord Båtsfjord and Bjørnevatn Altogether 308 school children 142 girls and 166 boys were included in the material The height, weight and number of subjects in the various age groups are given in Table 4 in the Appendix. Twenty-eight female and 30 male pupils from the Bjørnevatn school were studied twice a year (i.e April and October) during the period from April 1968 until April 1970 Thus altogether 6 examinations were performed in each of these subjects. The 58 subjects were sampled from two school classes i e 13 female and 16 male subjects from class A, and 15 female and 14 male subjects from another class (class B) Further information regarding these subjects is given in Table 5 and Table 6 in the Appendix.

Students

The student material included a total of 75 individuals. Of these 21 females and 20 males were physical education students. These subjects were also included in Study III The remainder of the student material 34 subjects, were all untrained and none of these took part in any regular physical activity Pertinent data describing the subjects are given in Table 7 in the Appendix.

Office-workers

The material consisted of 30 females and 57 males. This group of subjects was studied in 1961 being part of the material included in a cand real thesis at Oslo University (Hermansen 1964) None of these subjects were well-trained, although some of them regularly took part in outdoor activities, i e cross-country skiing, swimming etc Data characterizing the subjects are given in Table 7 in the Appendix.

Athletes.

Altogether 123 athletes were included in the material Of these, 85 were male and 38 were female athletes. All subjects were athletes of high national standard and most of them belonged to the Norwegian National Team in the different sports events. Some of them had been participants in several World Championships and Olympic Games These studies were performed during the period from 1967 to 1971 Pertinent data describing the subjects are presented in Table 8 and Table 9 in the Appendix.

Cross-sectional and longitudinal studies

The results of the cross-sectional and longitudinal studies concerning the changes in maximal oxygen uptake of schoolboys and schoolgirls are given in Fig 6–1 and Fig 6–2 The longitudinal studies, which included subjects from two school classes (class A and class B) showed that the variations in the mean values for maximal oxygen with age were almost the same in the two groups of subjects (i.e. school classes) Statistical analysis revealed no significant differences.

It will be seen from Fig 6–1 and Fig 6–2 that the mean values for maximal oxygen uptake in the longitudinal studies were approximately the same as those obtained in the cross-sectional studies. The differences were small and not statistically significant It should be emphasized that the number of subjects investigated in the cross-sectional studies varied from one age group to another Furthermore, the number of subjects in the longitudinal studies was greater in some age groups and lower in others than was the case in the cross-sectional studies.

Variation with sex and age

In order to study the variation in maximal oxygen uptake and related parameters (i.e. pulmonary ventilation heart rate) with sex and age in Norwegian subjects, a total of 429 untrained healthy subjects (i.e 186 females and 243 males) were examined. The results are presented in Fig. 6–3 to Fig 6–10 and in Table 4 and Table 7 in the Appendix.

As shown in Fig. 6–3 there is a pronounced increase in maximal oxygen uptake (l/min) during childhood in both sexes. In the female subjects, maximal oxygen uptake reached a peak value at the age of 14 to 16 years. From then on an almost rectilinear decrease with increasing age was observed. The maximal oxygen uptake was found to decrease from 2 62 l/min at the age of 16 to 1 81 l/min at the age of 54 years, i.e. 0 81 l/min (or 31%) in 38 years. Hence the maximal oxygen uptake in the female subjects decreased approximately 0 02 l/min (or 0 8%) per year In the male subjects, a somewhat different relationship between maximal oxygen uptake and age was observed The maximal oxygen uptake was found to increase almost rectilinearly from the age of 11 to the age of 16 years. From then on the maximal oxygen uptake increased at a slower rate, reaching a peak value at an age of approximately 25 years. Thereafter gradual decrease, as for the females, was observed Maximal oxygen uptake decreased from 3 96 l/min at the age of 25 years to 2 58 at the age of 54 years, i.e. 1 38 l/min (or 35%) in 24 years, representing about 0 06 l/min (or 1 5%) per year Thus, the annual decrease in maximal oxygen uptake with increasing age in the male subjects was almost twice as large as that of the female subjects.

The mean values for maximal oxygen uptake in the male subjects were higher than those of the female subjects in all age groups. The mean difference in the three youngest age groups (i.e. 11 12 and 13 years) was approximately 7% However due to a very large interindividual variation in both sexes, the difference was not statistically significant. The difference in the mean values for maximal oxygen

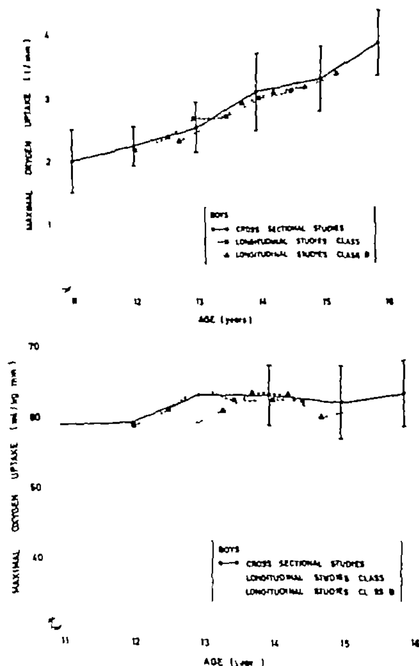


Fig. 6—1 Mean values for maximal oxygen uptake l/min (upper panel) and ml/kg x min (lower panel) in different age groups of schoolboys obtained in cross-sectional and longitudinal studies. Vertical bars denote ± one standard deviation.

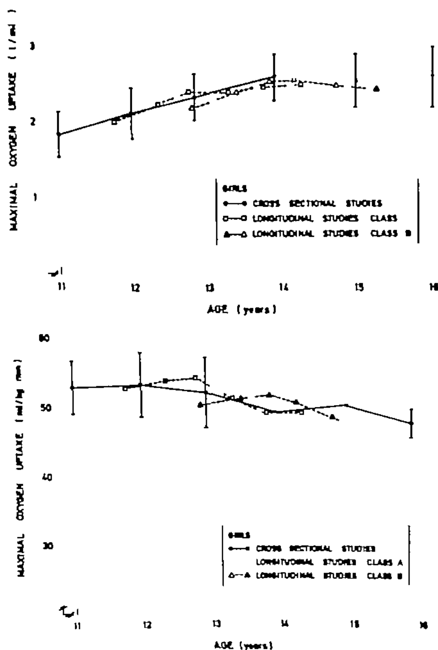


Fig. 6—2. Mean values for maximal oxygen uptake l/min (upper panel) and ml/kg x min (lower panel) in different age groups of schoolgirls, obtained in cross-sectional and longitudinal studies. Vertical bars den t ± on standard deviation.

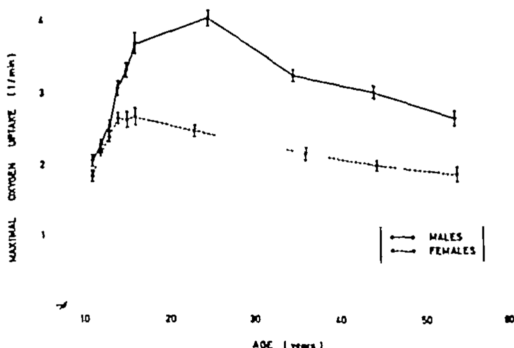


Fig. 6—5 Mean values for maximal oxygen uptake (l/min) in different age groups of female and male subjects. Vertical bars denote ± standard error of the mean.

uptake in the three next age groups, i.e 14 15 and 16 years was 15 5%, 21 4% and 31 9%, respectively The difference between the two sexes in the adult age groups was even larger The mean values for maximal oxygen uptake were 39 1%, 33 7%, 33 4% and 29 8% lower in the female than in the male subjects in the age groups 20—29 30—39 40—49 50—59 years, respectively All these differences were statistically significant

It should be emphasized that the maximal oxygen uptake and the body weight varied considerably from one individual to another The large variation in these two parameters was most markedly demonstrated in the age group with the largest number of subjects (Fig 6—4) i.e 12 year-old boys and girls. Maximal oxygen uptake was in these subjects found to vary between 1 48 to 3 13 l/min. The body weight varied between 27 6 and 61 4 kg

The variation in maximal oxygen uptake after correction for differences in body weight showed a somewhat different picture that that described above for the maximal oxygen uptake in l/min. The maximal oxygen uptake (ml/kg x min) in the female subjects reached a peak value at the age of 12 years. From then on, a gradual decrease with increasing age was observed For the male subjects, however the peak value was observed at the age of 13 years and the values were practically unchanged in the age groups 14 15 and 16 years However the mean values for the male

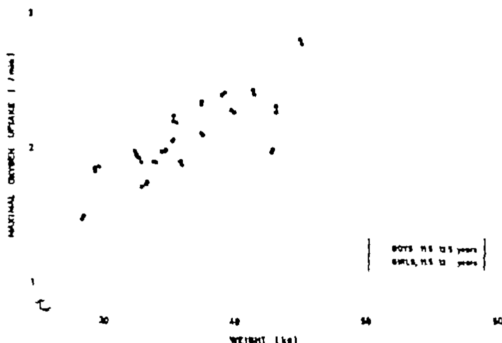


Fig. 6—4. Individual values for maximal oxygen uptake (l/min) of schoolboys and schoolgirls, 11.5 to 12.5 years of age in relation to body weight.

students were significantly lower than for the 16 year-old boys. In the case of the women, the mean values for maximal oxygen uptake continued to decrease with increasing age

It should also be noted that the rate of decrease in maximal oxygen uptake was different in young (i.e. 12 to 23 years in the women, and 16 to 34 years in the men) and older subjects. For the female subjects the maximal oxygen uptake decreased from approximately 54 ml/kg x min at the age of 12 to about 38 ml/kg x min at the age of 23 i.e 16 ml/kg x min (or about 30%) in 11 years. Hence the rate of decrease was approximately 2 5% per year For the male subjects, maximal oxygen uptake was found to decrease from approximately 63 ml/kg x min at the age of 16 to about 41 ml/kg x min at the ag of 34 years, i.e. 22 ml/kg x min (or 35%) in 18 years. Thus the rate of decrease in the young male subjects was approximately the same as that observed in the young women (i.e. 2% per year)

The rate of decrease in maximal oxygen uptake in the older subjects was, however lower than in the younger subjects. The mean values for maximal oxygen uptake in the female subjects decreased from approximately 38 ml/kg x min at the age of 23 to about 29 ml/kg x min at the age of 54 years, i.e 9 ml/kg x min, or 24% in 31 years (0 8% per year) In the male subjects, the mean values decreased from

approximately 42 ml/kg x min at the age of 34 years to 36 ml/kg x min at the age of 54 years, i.e. 8 ml/kg x min, or 19% in 20 years (0.9% per year). Hence the rate of the decrease in maximal oxygen uptake with increasing age in the older subjects was found to be almost the same in both female and male subjects. Furthermore, from the results presented in Fig. 6–5 it seems that the rate of decrease in the mean values for maximal oxygen uptake has two phases: a rapid phase in the younger subjects, and a slower one in the older subjects.

In spite of the large variation in maximal oxygen uptake between female and male subjects, no significant difference in the values for heart rate during maximal exercise was observed. However, the mean values were found to decrease with increasing age in both sexes (Fig. 6–6). The average decrease from the age of approximately 15 to 55 years (i.e. 40 years) was 33 and 27 beats/min, i.e. 0.83 and 0.68 beats/min each year, for the females and the males respectively. The changes in the mean values for pulmonary ventilation during maximal exercise and the ventilatory equivalent (i.e. ventilation per liter of oxygen consumed) are given in Fig. 6–7 and Fig. 6–8 respectively. The variations in the maximal pulmonary ventilation were closely related to the changes in the maximal oxygen uptake and consequently the ventilatory quotient (Fig. 6–8) changed very little with age. A small decrease, however, in the ventilatory quotient with increasing age was observed.

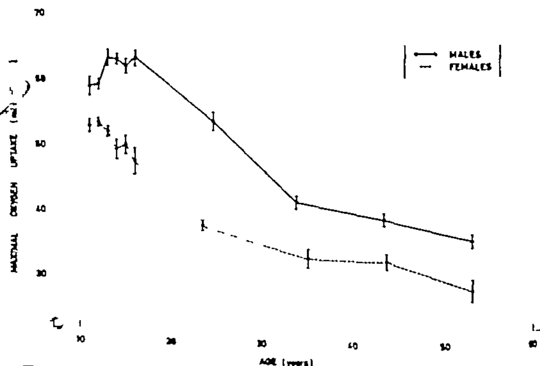


Fig. 6–5 Mean values fo maximal oxygen uptake (ml/kg x min) in different age groups of mal and female subjects. Vertical bars denote ± standard error of the mean.

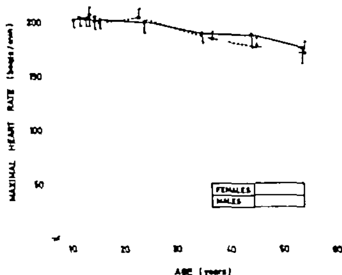


Fig. 6–6. Mean values for heart rate (beats/min) during maximal exercise in different age groups of male and female subjects.

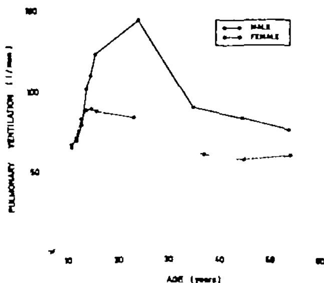


Fig. 6–7 Mean values fo pulmonary ventilation (l/min BTPS) during maximal exercise (i.e. running o bicycling) in different age groups of mal and female subjects.

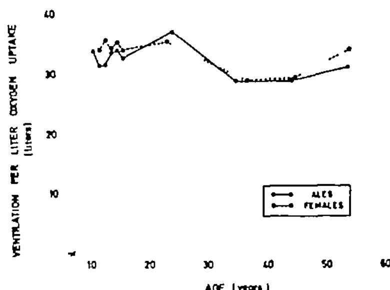


Fig. 6—8 Mean values for the pulmonary ventilation per liter of oxygen consumed during maximal exercise (i.e running or bicycling) in different age groups of male and female subjects.

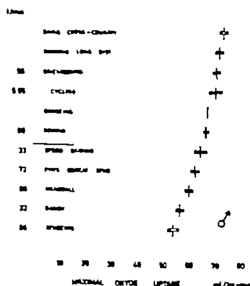


Fig. 6—9 Mean values for maximal oxygen uptake in ml/kg x min of male athletes in different sports events. The horizontal small bars at the top of the large bars denote standard error of the mean. To the left mean values for maximal oxygen uptake in l/min are also given. Mean values for maximal oxygen uptake of untrained students are also included.

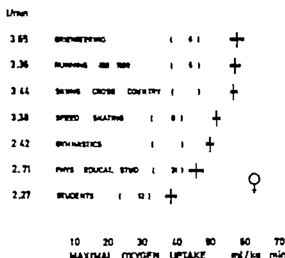


Fig. 6–10. Mean values for maximal oxygen uptake in ml/kg x min of female athletes in different sports events. The horizontal small bars at the end of the large bars denote standard error of the mean. To the left mean values for maximal oxygen uptake in l/min are also given. Mean values for maximal oxygen uptake of untrained students are also included.

Maximal oxygen uptake in athletes

In order to obtain an idea of the upper limit of the values for maximal oxygen uptake in Norwegian subjects, groups of female and male athletes in different sports events were examined The results are presented in Fig 6–9 and Fig 6–10 and in Table 8 and Table 9 in the Appendix.

It will be seen that there was a large difference in the mean values for the different groups, even in well-trained subjects. The highest val es were observed in athletes who took part in endurance events (i.e. cross-country skiing orienteering long distance running or bicycling) There were 6 male subjects with a maximal oxygen uptake of more than 6 l/min. The highest value for maximal oxygen uptake in the present investigation 6 64 l/min (72 ml/kg x min) was obtained in a top speed skater (B T) who came third in 1500 m speed skating at the Olympic Games in Grenoble 1968 The second highest value 6.26 l/min (67 ml/kg x min) was observed in a rower (F.H.) who was one of the two sculler rowers who came second in the Olympic Games in Munich 1972. Subject F.S.H., a cross-country runner (orienteering) of high national standard had a maximal oxygen uptake of 82 ml/kg x min. He was the only subject in the present investigation who exceeded 80 ml/kg x min.

The highest maximal oxygen uptake in the female athletes was found in two cross-country skiers, both belonging to the team which won the gold medal in the relay race (3 x 5 km) t the Olympic Games in Grenoble 1968 B.M.L. had a

The value for maximal oxygen uptake is dependent upon the body size of the subject. Consequently, the height and weight of the subjects are important parameters when deciding whether or not the material is representative. However, recent studies of height and weight of representative samples of the Norwegian population are not available. The control material of school children was sampled from 9 different schools in Oslo (i.e. Oslo and suburbs). The investigation was performed by Hermansen and Skaset in 1970 (unpublished results) and included a total of 1076 students between 7 and 18 years of age. The number of subjects in most age groups (i.e. one year) was approximately 50 male and 50 female subjects. The untrained adult subjects were studied in connection with the X-ray examination in the county of Østfold (i.e. the southeastern part of Norway) in 1965 (Waaler 1972). This material included approximately 2500 male and female subjects in each age group (i.e. 5 years). Unfortunately the values for standard deviation and standard error of the mean were not calculated in this study.

The mean values for height and weight in the different groups of subjects in the present investigation are compared with the values obtained in the above mentioned studies (Hermansen and Skaset 1970, Waaler 1972) (Fig. 6–11 and Fig. 6–12). The height/weight relationship in the same subjects is shown in Fig. 6–13. It will be seen (Fig. 6–11 and Fig. 6–12) that the mean values for height and weight are somewhat lower in the school children from Finnmark than in school children of comparable age from Oslo. However, statistical analyses revealed no significant differences. There was also a tendency toward somewhat higher values for the body weight at a certain height in the school children from Finnmark compared with school children in Oslo. The differences, however, were small and not statistically significant. Furthermore, it will also be seen (Fig. 6–11 and Fig. 6–12) that in the adult subjects the mean values for body weight were somewhat lower, and the mean values for the body height were somewhat higher in the subjects of the present investigation compared with the results obtained by Waaler (1972). The height of the athletes was approximately the same as for the untrained subjects, while the mean values for the body weight were somewhat higher in most groups of athletes, compared with untrained subjects. The mean value for the body weight of the female gymnasts, however, was lower than in untrained subjects of comparable age.

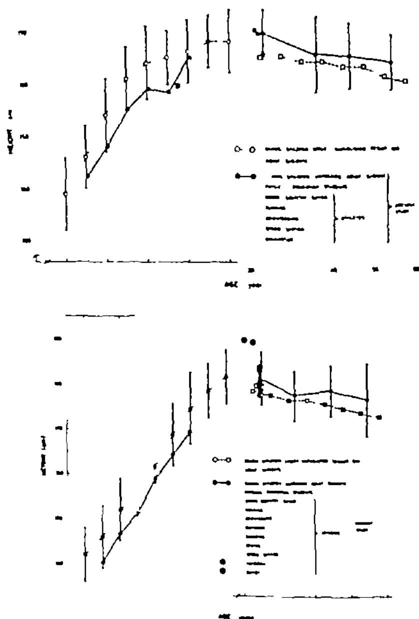


Fig. 6–11 Mean val es for the body height in rel tion to age in female subjects (upper panel) and male subjects (lower panel) in the present investigation. Corresponding mean val es for school children in Oslo (o—o) and adults subjects (□—□) are given for comparison. Vertical lines denote ± one standard deviation.

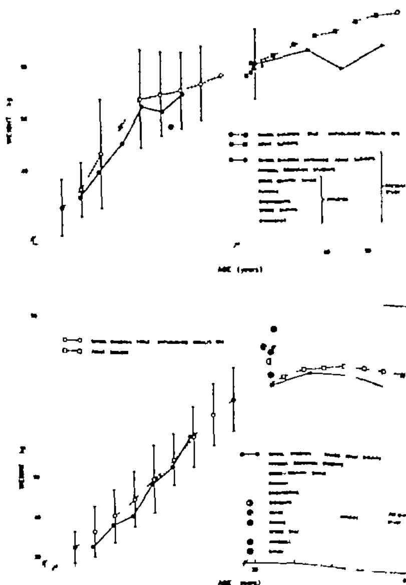


Fig. 6—12. Mean values for the body weight in relation to age in female subjects (upper panel) and mal subjects (lower panel) in the present investigation. Corresponding mean values for school children in Oslo (o—o) and adult subjects (□—□) are given for comparison Vertical lines denote ± one standard deviation.

Fig 6—15 The height/weight relationship in female subjects (upper panel) and male subjects (lower panel) in th present investigati n Corresponding values for school children in Oslo (o—o) are given for comparison.

CHAPTER VII

GENERAL DISCUSSION

Maximal oxygen uptake expressed in liters per minute is a measure of the maximal rate of aerobic energy liberation within the body (i.e. the maximal aerobic power). It is also a measure of the *functional capacity of the respiratory and circulatory* systems (Taylor et al. 1955, Mitchell et al. 1958a, Rowell 1962, Saltin et al. 1968). Maximal oxygen uptake expressed in milliliters per kilogram body weight is a measure of the maximal amount of oxidative energy available to move one kilogram of the body weight from one place to another in a certain length of time. Maximal oxygen uptake is also widely used *as a measure of physical fitness. A great number* of factors are known to cause a decrease in maximal oxygen uptake (Åstrand 1967). The only factors which have been shown to increase the maximal oxygen uptake are physical training (Robinson and Harmon 1941, Knehr et al. 1942, Saltin et al. 1968, Ekblom et al. 1968) and an increase of the oxygen tension in the inspired air (*Margaria et al. 1961, Wyndham et al. 1970). In a recent study* by Ekblom et al. (1972) maximal oxygen uptake was also reported to increase after increasing the red cell volume by reinfusion of red blood cells.

Investigations of various groups of subjects in many countries have shown that the mean values for maximal oxygen uptake may vary considerably from one individual to another and also *from one group* of subjects to another (Robinson 1938, Åstrand 1952, Mitchell et al. 1958a, Åstrand 1960, Hettinger et al. 1961, Rodahl et al. 1961, Andersen and Hermansen 1965, Hermansen and Andersen 1965, Binkhorst et al. 1966, Sprynarova 1966, Andersen 1967b, Cumming 1967, Ekblom and Gjessing 1968, Ikai et al. 1970, Davies 1972, Davies et al. 1972, Dehn and Bruce 1972, Eriksson 1972, Ikai and Kitagawa 1972 and others). In tables 7–1 to 7–4 the mean values for maximal oxygen uptake (ml/kg x min) in the present investigation are compared with results obtained in some other countries. It will be seen from Tables 7–1 and 7–2 that th mean values for maximal oxygen uptake in the school children of the present investigation are higher than in all other studies reported in the literature, although the study by Åstrand (1952) of school children in Stockholm reported almost the same mean values in most age groups of both sexes as those obtained in the present investigation. With regard to the maximal oxygen uptake in boys (Table 7–1) it is worth noticing that the mean values obtained in USA (Robinson 1938), Canada (Cumming 1967) and J pan (Ikai et al. 1970) are almost identical. However all these values are significantly lower than the values for the Scandinavian school children (Åstrand 1952, present investigation). The lowest values for maximal oxygen ptake in boys are found in the USA (Rodahl et al. 1961). The mean

Table 7–1 Mean values for maximal oxygen uptake (ml/kg x min) in different age groups of boys from various populations.

Age years	U.S.A. Robinson 1938	SWEDEN Åstrand 1952	U.S.A. Rodahl et al. 1961	CANADA Cumming 1967	JAPAN Ikai et al. 1970	NORWAY present study
11	5	57	–	–	52	59
1	50	56	32	47	53	59
13	48	57	–	–	5	63
14	47	59	53	49	51	63
15	48	60	–	–	50	62
16	50	59	32	52	55	63

Table 7–2. Mean values for maximal oxygen uptake (ml/kg x min) in different age groups of girls from various populations.

Age years	SWEDEN Åstrand 1952	U.S.A. Rodahl et al. 1961	CANADA Cumming 1967	JAPAN Ikai et al. 1970	NORWAY Present study
11	53	–	–	46	53
12	51	30	42	44	54
13	49	–	–	43	52
14	48	34	38	38	50
15	45	–	–	40	50
16	47	23	39	36	48

Table 7–3. Mean values for maximal oxygen uptake (ml/kg x min) in different age groups of male subjects from various populations.

Age years	U.S.A. Robinson 1938	U.S.A. Mitchell et al. 1958a	SWEDEN Åstrand 1960	U.S.A. Hettinger et al. 1961	CANADA Cumming 1967	NORWAY Present study
20–29	55	45	52	30	44	54
30–39	41	39	40	29	38	42
40–49	40	35	39	26	38	39
50–59	37	32	33	25	36	36

Table 7–4. Mean values for maximal oxygen uptake (ml/kg x min) in different age groups of Swedish and Norwegian women

Age years	SWEDEN Åstrand 1960	NORWAY Present study
20–29	40	38
30–39	37	34
40–49	33	33
50–59	28	29

for maximal oxygen uptake of the girls in the present investigation were somewhat higher than those obtained in girls of comparable age in Stockholm (Åstrand 1952) However as in the case of the boys, the mean values for the maximal oxygen uptake in the Scandinavian girls were found to be significantly higher than the values obtained in Canada (Cumming 1967) and Japan (Ikai et al 1970) On the other hand the mean values from the two latter studies in Canada and Japan are significantly higher than the corresponding mean values obtained in American girls (Rodahl et al. 1961)

In table 7–3 and Table 7–4 the mean values for maximal oxygen uptake of adult subjects in the present investigation are compared with corresponding mean values from some other investigations. Considering the possible differences in methodology number of subjects investigated in the various age groups of the different studies, and the time difference (the earliest study was performed in 1938) surprisingly small differences exist between samples of the male population in Boston, Stockholm, Dallas, Winnipeg and Oslo The Philadelphia group (USA) studied by Hettinger et al (1961) is the only group in which significantly lower values than those of the above mentioned studies are reported. Only scanty information is available concerning maximal oxygen uptake in adult female subjects. The mean values for Swedish females (Åstrand 1960) are almost identical to the mean values obtained in the present study

Although the mean values for maximal oxygen uptake are very similar in many groups of subjects from different countries, it is evident from the results presented above (Table 7–1 to 7–4) that large differences exist between the highest and the lowest values reported in the literature both for children and adult subjects. The most pronounced difference is found between the values reported by Rodahl et al. (1961) and those of the present study for 16 year-old subjects (boys and girls) The average maximal oxygen uptake in the American 16 year-old boys was 32 ml/kg x min, compared with 63 ml/kg x min in the present investigation The corresponding value for 16 year-old American girls was 23 ml/kg x min and for the Norwegian girls the value was 48 ml/kg x min

It is not possible, on the basis of the results of the present investigation to explain the large differences between the mean values for maximal oxygen uptake in the various groups investigated. It is also difficult to explain the large intragroup variability

With regard to the differences in the mean values for maximal oxygen uptake as shown in Table 7–1 to Table 7–4 it should be emphasized that only a small number of subjects were studied in the various age groups of the different investigations. Furthermore most of the studies were not based on a representative sample of the population investigated. Thus there might be some doubts as to whether or not the mean values are representative for the population from which the subjects were selected On the other hand it was shown by Grimby et al. (1970) that the mean value for maximal oxygen uptake in a large and representative group of 54 year-old male subjects was almost the same as the mean values obtained in many other studies with only a few subjects investigated These authors (Grimby et al. 1970) concluded that the results of their study with representative sampling increased the validity of the conclusions which might be drawn from earlier studies with no representative sampling

In the studies of Andersen et al (1961) Hermansen and Ekblom (1966) and Ekblom and Gjessing (1968) it was shown that the mean values for maximal oxygen uptake in the nomadic Lapps in North Norway were higher in most age groups, both in the female and male subjects, than the corresponding mean values for the inhabitants of the Easter Islands (the Pascuans) On the basis of these results it might be questioned as to whether or not part of the observed differences in maximal oxygen uptake between groups of subjects from different countries (Tables 7–1 to 7–4) could be due to environmental factors or to racial differences brought about by the process of natural selection. However studies by Robinson et al. (1941) on white and American negro sharecroppers revealed no significant difference. Rodahl et al. (1961) studying white and nonwhite school children in Philadelphia found no evidence suggesting a systematic racial difference in physical work capacity Recent studies by Davies et al (1972) also seem to indicate that if differences between ethnic groups do exist they are certainly smaller than the large interindividual variations observed in most studies.

It is, however well known that variations in the level of physical activity may influence the maximal oxygen uptake to a large extent (Robinson and Harmon 1941 Knehr et al. 1942 Saltin et al 1968) Thus, it is reasonable to suggest that part of the differences in maximal oxygen uptake as shown in Tables 7–1 to 7–4 must be attributed to variations in the level of physical activity Variations in the methods and procedures employed for the determination of the maximal oxygen uptake are another factor which may probably explain part of the observed differences. With regard to the large differences in the individual values for maximal oxygen uptake within the groups, variations in the level of physical activity are probably one important factor (Saltin et al 1968) However according to the investigations on monozygous and dizygous twins by Klissouras (1971) the intragroup variability is almost entirely genetically determined

Several factors are known to influence the obtained values for maximal oxygen uptake n human subjects (Rowell 1962 Åstrand 1967) The type of work used for the determination is one essential factor (Taylor et al 1955) and the mass of muscles activated during the work is another important factor (Christensen 1931 Asmussen and Hemmingsen 1958 Åstrand and Saltin 1961b Stenberg et al 1967) Since the treadmill and the bicycle are the two most frequently employed ergometers, it is mportant to compare the physiological responses to exercise using

these two testing devices. In the present investigation it was shown that "ski-walking" with poles on the treadmill yielded higher values for maximal oxygen uptake than those obtained during uphill running. Uphill running (inclination 3° or more) on the other hand gave higher values for maximal oxygen uptake than those obtained during maximal bicycle exercise (50 rpm). It was also shown that the values for oxygen uptake during maximal uphill running were higher than those obtained during maximal running at no inclination. The differences in maximal oxygen uptake between uphill running, on the one hand and bicycling and running at no inclination on the other were almost the same

The present investigation also showed that the pedal frequency during the bicycling, and the step frequency during treadmill running are factors which modify the results to a certain extent. Maximal oxygen uptake was found to be higher during bicycling at 60 and 70 rpm than during bicycling with pedal frequencies of 40 or 50 rpm A pedal frequency of 80 rpm however seemed to be too high to obtain the highest possible oxygen uptake The step frequency during treadmill running at no inclination is about 190–200 steps/min If the inclination of the treadmill is increased to about 3 the step frequency is reduced to approximately 160 steps/min. Thus, both during bicycling and running the contraction frequency of the working muscles seems to be a factor which influences the maximal oxygen uptake

The finding of the present investigation indicating a higher oxygen uptake during arm plus leg exercise confirm earlier observations by Åstrand (1952) and on one subjects studied earlier by Christensen and Hogberg (1950) showing that skiing with poles gave higher values for oxygen uptake than those obtained during running Taylor et al (1955) found a significant increase in maximal oxygen uptake in one subject, who while running on the treadmill also worked an arm ergometer Furthermore Andersen et al. (1961) found higher values for maximal oxygen uptake during skiing than during bicycling In a recent study by Kamon and Pandolf (1972) laddermill climbing (i.e arm and leg exercise) was found to give higher values for maximal oxygen uptake than those obtained during bicycling The results of the present investigation are also in agreement with the studies by Faulkner et al. (1971) and Miyamura and Honda (1972) All these investigations seem to indicate that the values for maximal oxygen uptake increase with the increase of the mass of muscles activated during work.

The explanation of the above described differences is u clear However it has been known for a long time that the motor nits in human skeletal muscle vary widely in size. Moreover the full significance of these observations did not become apparent until after the investigation by Henneman et al. (1965) indicating that the excitability of the units is an inverse function of their size. Furthermore, it has been shown that human skeletal muscle consists of two different fibre types. These fibres have been classified as type I and type II (Dubowitz and Pearse 1960 Morris 1968) red and whit fibres (Edström and Nyström 1969 Edström and Ekblom 1972) or slow twitch (ST) and fast twit h (FT) fibres (Gollnick et al. 1972) It is also known that the different fibre types vary with regard to both contractile and metabolic properties (Barnard et al. 1971 Burke et al. 1971) Due to the differences in th excitability of the motor units, and to differences in contractile and metabolic properties, it has been assumed (Jorfeldt 1970 Karlsson 1971) that it is mainly the

small motoneurons (α 2) and slow twitch fibres (i.e red muscle fibres) which are activated during exercise at low work intensities and that the contribution of large motoneurons (α 1) and fast twitch fibres (i.e. white muscle fibres) increases with increasing work loads. It is conceivable that the total number of motor units, and probably also the total number of red muscle fibres activated are larger during for instance "ski-walking than during bicycling

If it is assumed that the muscle mass ratio between the arms and shoulders, and the legs and hips is approximately 1:6 a 15 percent increase in the maximal oxygen uptake during arm plus leg exercise should be expected The increase observed in the present investigation was, however only 2 3 percent Although the muscles of the arms and shoulders are also activated to a certain extent, during running, it is reasonable to suggest that the increase in oxygen uptake is less than what should be expected from the increase in the mass of muscles activated during "ski-walking

However to what extent a larger muscle mass as such may explain the observed differences in maximal oxygen uptake is unclear as the values for maximal oxygen uptake during swimming (i e arm plus leg exercise) are shown to be lower than the values obtained during bicycling or running (Holmér 1972) Furthermore, there was no difference in oxygen uptake (Åstrand and Saltin 1961b) or cardiac output (Stenberg et al 1967) when leg exercise (bicycling) was compared with arm plus leg exercise (simultaneously cranking and bicycling) It is, however possible that simultaneously cranking and bicycling or running at high speeds and low grades are technically so difficult that this limits the performance before the maximal oxygen uptake is reached

With regard to the differences in maximal oxygen uptake between uphill running bicycling Glassford et al (1965) suggested that local muscular fatigue ced in the muscles of the lower extremities during maximal bicycling might ~~li~~t the work performance, and that this happens before the oxygen transport system is maximally taxed The results of Hoes et al (1968) which showed that the peak load during a pedal revolution during bicycling is twice as high as the actual load setting may be interpreted to support this view With regard to the contraction frequencies of the muscles during work it is also worth noticing the studies of Folkow and co-workers (Folkow et al 1970 Folkow et al 1971) showing that there is almost no inflow of blood during the contraction phase whereas each contraction is accompanied by a large increase in the venous outflow It was also shown that the blood flow through the muscle and the venous return to the heart was markedly affected by the contraction frequency Thus, from the results of the present investigation and from those of others, there seems to be an optimal relationship between the force of each muscle contraction and the contraction frequency of the muscles (viz step frequency during running and pedal frequency during bicycling) and that this is an important factor for increasing the oxygen uptake to the highest possible level

It is known that the effect of warm-up (Taylor et al 1955) or a hot environment (Rowell et al 1964) are factors which may influence the maximal oxygen uptake However in the present investigation all subjects performed almost the same warm-up program and all experiments were performed at approximately the same environmental temperature (i.e about 20 C) Therefore, it is not likely that these factors have influenced the results of the present investigation.

Although no definite explanation can be given for the above described differences in maximal oxygen uptake during different types of work, there are some practival implications to be noted To ensure that the highest possible oxygen uptake is reached arm plus leg exercise (i e "ski-walking") should be used. However it should be emphasized that the values for maximal oxygen uptake obtained during "ski-walking and uphill running were almost the same For the bicycle ergometer test a pedal frequency of 60 to 70 rpm is recommended Furthermore it should be stressed that the term maximal oxygen uptake refers only to the type of exercise used for the test. A strict comparison of the values for maximal oxygen uptake therefore requires strictly standardized conditions for the determinations.

The question concerning possible limiting factors has been debated for at least 50 years. Hill and Lupton in 1923 stated that it is still an open question whether the oxygen uptake during maximal exercise is limited by the heart or by the lungs. Christensen et al. (1934) listed 5 main factors influencing the oxygen supply to the muscles. These were 1) pulmonary ventilation 2) rate of diffusion of oxygen from alveolar air to the blood 3) cardiac output 4) the rate of blood flow through the muscles, and 5) the oxygen diffusion from the muscle capillaries to the cells. In later studies (Åstrand 1952 Taylor et al 1955 Mitchell et al 1958a, Åstrand and Saltin 1961b Rowell 1962 Saltin 1954 Holmgren 1967 Kaijser 1970 Karlsson 1971 Gollnick et al. 1972 and others) these and other factors have been discussed The fact that the identification of the possible limiting factors for maximal oxygen uptake is a complex problem to analyze is at least partly illustrated by the fact that the transport of oxygen from air to the mitochondria in the cells includes two convective systems (i.e ventilation and circulation) and two diffusing systems (i e. from the alveolar air to the blood and from the capillaries to the mitochondria) On the basis of the results of the present investigation some of the different factors which have been considered to influence the maximal oxygen uptake will be discussed

The pulmonary ventilation is not considered to be a limiting factor for the maximal oxygen uptake under normal conditions and in young healthy subjects. The results of the present investigation (Fig 3–5 and Fig 3–6) may be interpreted as supporting this view It was shown in Fig 3–6 that with increasing pedal frequency during maximal bicycle exercise the oxygen uptake levelled off the pulmonary ventilation however continued to increase. Furthermore, maximal oxygen uptake was higher during uphill running than d ring bicycling (50 rpm) while the pulmonary ventilation was the same (Fig 3–5) These results are in agreement with several earlier studies, reaching the same conclusion (Åstrand 1952 Mitchell et al. 1958a, Rowell 1962, Saltin et al. 1968)

The concept that pulmonary ventilation does not represent a limiting factor for the transport of oxygen during maximal exercise is also supported by the following evidence The pulmonary ventilation normally bears an approximately linear relationship to the oxygen uptake (or work load) up to some critical level representing approximately 60–70 percent of the individual s maximal oxygen uptake. Beyond this critical level the ventilation increases out of proportion to tl oxygen consumption (Asmussen and Nielsen 1946) The increase in pul ventilation is brought about by a progressive increase in both tidal vo

respiratory rate. The former increases more steeply than the latter during light to moderate loads. The respiratory adjustment to further increase in oxygen uptake however is almost entirely due to an acceleration in the rate of breathing (Christensen 1932) However neither of these factors (i.e. tidal volume or respiratory rate) reach their highest possible values during maximal exercise According to Dripps and Comroe (1947) and Freedman (1970) healthy subjects utilize less than 70 percent of their maximal voluntary volume during work loads demanding maximal oxygen uptake On the other hand it should be emphasized that the maximal voluntary volume is defined as the highest possible ventilation during 10–15 seconds. The highest possible ventilation during 4 min is shown to be only 70–75 percent of the maximal voluntary volume (Freedman 1970) The relationship between the pulmonary ventilation and the pulmonary circulation is another important factor During exercise there is an increase in the overall ventilation/perfusion ratio and the distribution of air and blood to the different parts of the lungs becomes less uneven (West and Dollery 1960) During maximal exercise there is also an increase in oxygen tension and a decrease in carbon dioxide tension in the alveolar air (Asmussen and Nielsen 1958) Thus, according to the above mentioned studies, the pulmonary ventilation seems to be large enough to secure an adequate alveolar oxygen tension even during maximal exercise

Normally the diffusion of oxygen from the alveolar air to the blood is not considered a limiting factor for maximal oxygen uptake in young subjects (Holmgren and Åstrand 1966 Saltin et al 1968). This view is based at least partly evidence from measurements of diffusion capacity (Saltin et al. 1968) arterial ygen tension (Mitchell et al. 1958) and oxygen saturation of arterial blood a and Linderholm 1958 Mitchell et al 1958 Asmussen and Nielsen 1960 owell 1964 Saltin et al 1968) during submaximal and maximal exercise. On the other hand there is evidence which suggests that the diffusing capacity of the lungs might be a limiting factor during maximal exercise in older subjects (Cohn et al 1954) This finding may at least partly explain the reduction in maximal oxygen uptake with age which was observed both in female and male subjects in the present investigation.

Oxygen uptake at rest and during exercise is determined by the product of cardiac output and the arteriovenous oxygen difference Thus, if it is considered that neither pulmonary ventilation nor the diffusion of oxygen from the alveolar air to the blood limits the oxygen uptake during maximal exercise two main possibilities seem to exist First, that the maximal oxygen uptake may be limited by the capacity to transport oxygen from the lungs to the active tissues. This could be due to either a low oxygen carrying capacity of the blood (i.e a low hemoglobin concentration) or an inability to increase the cardiac output or a combination of both factors. The cardiac output, in turn could be limited by a low maximal heart rate, a low maximal stroke volume or a combination of both Secondly that the maximal oxygen uptake may be limited by an inability of the tissues to extract more oxygen from the blood i.e. a low arteriovenous oxygen difference. This could be due to an inefficient distribution of the cardiac output or a low capacity of the active tissues to consume the oxygen brought to them

A low oxygen carrying capacity of the blood (i.e. a low hemoglobin concentration) is the first factor to be analyzed Cross-sectional analysis of the

results from the present investigation showed a close association between hemoglobin concentration and maximal oxygen uptake when the whole material (i.e. school children and students) was considered However this association could not be confirmed when the correlation analysis was performed for each of the subgroups separately (i.e. schoolgirls, schoolboys, female and male students) Furthermore it was shown (Fig 4–2) that at the same maximal oxygen uptake (for instance 2 5 l/min) the hemoglobin concentration might vary between 11 5 and 15 0 g/100 ml. On the other hand at the same hemoglobin concentration (for instance 13 6 g/100 ml) maximal oxygen uptake might vary between 1 8 and 5 2 l/min. These results show that the same maximal oxygen uptake may be transported with quite different hemoglobin concentrations, and that subjects having the same hemoglobin concentration might have different maximal oxygen uptakes. These observations are in agreement with several previous investigations (Åstrand 1952 von Döbeln 1956 Buskirk and Taylor 1957) It was shown by von Döbeln (1956) that the correlation coefficient between total hemoglobin content and maximal oxygen uptake was 0 725 However the correlation coefficient between fat free body weight and maximal oxygen uptake was even higher (i.e. 0 756) Thus the partial correlation coefficient between total hemoglobin content and maximal oxygen uptake was only 0.246 On the basis of these results, von Döbeln (1956) concluded that the main reason for the close correlation between the total hemoglobin content and the maximal oxygen uptake was that the total hemoglobin content is related to the body size. The results of the present investigation may be interpreted to support the conclusion of von Döbeln (1956)

The only suggestion of an impaired oxygen transport in subjects with low hemoglobin concentration is given by Sproule et al. (1960) These authors observed lower values for maximal oxygen uptake in 9 anemic men (hemoglobin concentration 5 2 – 9 3 g/100 ml) than the corresponding values for healthy men of comparable age studied by Mitchell et al. 1958a, b Whether such a comparison is relevant or not, might be questionable There are several points in the study of Sproule et al. (1960) which should not be overlooked Maximal oxygen uptake was neither obtained in all subjects (i.e only in 6 of the 9 subjects) nor measured fter restoration of the hemoglobin concentration. Since all 9 subjects were included in the study the mean value for maximal oxygen uptake is too low Furthermore it is interesting to note that the anemic subjects were able to run on the treadmill at the same speed and duration as that of the healthy subjects studied by Mitchell et al (1958a) The possible explanation that anaerobic processes might have been taxed to a larger extent in the anemic subjects, is not supported by the fact that the blood lactate concentrations were lower in the anemic subjects than in the healthy ones. Finally it should be emphasized that the values for maximal oxygen uptake were not corrected for d fferences in body size, and that at least 3 of the subjects studied by Sproule et al (1960) had body weights of 56.2 55.2 and 49 4 kg Thus, it appears that the maximal oxygen uptake in the anemic subjects, if expressed in ml/kg x min, might possibly be the same, or almost the same, as that observed in the healthy subjects.

The results discussed above were all obtained in cross-sectional Information about the effect of variation in the hemoglobin on the

oxygen uptake in longitudinal studies, on the other hand is limited The results of the longitudinal experiments in the present investigation showed that in the same subject hemoglobin concentration may increase (or decrease) considerably while maximal oxygen uptake may remain unchanged increase or decrease In some subjects the hemoglobin concentration remained at the same level while maximal oxygen uptake increased considerably Thus, it may be concluded that the cross-sectional and longitudinal analysis of the present investigation showed no consistent relationship between hemoglobin concentration and maximal oxygen uptake.

These observations are in agreement with the results of several previous investigations. Saltin (1964) for instance found no change in maximal oxygen uptake after thermal dehydration which increased the hemoglobin concentration by 8 percent and reduced the plasma volume by 14 percent. Robinson et al. (1966) found no change in maximal oxygen uptake after transfusion of one liter of blood. Furthermore Saltin et al (1968) observed only small changes in the relative or total hemoglobin content after bed rest or training Maximal oxygen uptake, however varied considerably

Several investigations have on the other hand showed a reduction in the physical performance capacity accompanying the lowered hemoglobin concentration after blood loss (Karpovich and Millman 1942 Balke et al 1954 Rowell et al 1964 Ekblom et al 1972) However with regard to the effect of the reduced hemoglobin concentration on the capacity to transport oxygen, the various studies have yielded conflicting results. Thus, Rowell et al (1964) for instance, found that a reduction in hemoglobin concentration of 14 percent due to repeated phlebotomies over an eight-day period gave almost no reduction (4 percent) in maximal oxygen uptake. Ekblom et al (1972) on the other hand showed that hemoglobin concentration decreased, on the average 13 percent after blood loss of 800 ml in 3 subjects (group I) and 18 percent after a blood loss of 1200 ml in 4 subjects (group II) Maximal oxygen uptake decreased to the same extent, i.e. 13 and 18 percent in group I and group II respectively In the same experiment it was shown that after reinfusion of red blood cells both hemoglobin concentration and maximal oxygen uptake increased.

Thus, according to the above mentioned studies, variations in the total blood volume, plasma volume or red cell volume do not seem to give any consistent effect on the maximal oxygen uptake. Furthermore, a comparison of the results obtained in these studies, which included transfusion of whole blood (Robinson et al 1966) or red blood cells (Ekblom et al. 1972) phlebotomies (Rowell 1962 Ekblom et al. 1972) dehydration (Saltin 1964) and bed rest and training (Saltin et al. 1968) might be questionable. All the different experimental conditions, as such, might have effected the physical performance capacity of the subjects and thus indirectly also the transport of oxygen.

If the same amount of oxygen is transported from the lungs to the muscles per unit of time with low and high hemoglobin concentrations, compensatory mechanisms must be involved The results of the present study as well as several other investigations (Sproule et al. 1960 Murray et al. 1962 Åstrand et al 1964 Grimby et al. 1966 Crowell and Smith 1967 and others) indicate that variations in the hemoglobin concentration may be compensated by changes in the cardiac output or arteriovenous oxygen difference, or by a combination of both factors.

The results from two measurements on the same well-trained athlete (A.H.) performed with an interval of 5 years, indicate that a fall in the hemoglobin concentration may be compensated for by an increase in cardiac output. A.H. (no 12) was studied the first time in 1962 by Åstrand et al. (1964) and the second time in 1967 (Ekblom and Hermansen 1968). Maximal oxygen uptake was 5.39 l/min in 1962. This oxygen uptake was achieved by a cardiac output of 28.8 liter/min and an arteriovenous oxygen difference of 187 ml per liter of blood. The hemoglobin concentration was 16.5 g/100 ml. This gives a cardiac output of of 5.34 liters per liter of oxygen consumed. When the same subject visited the same laboratory 5 years later (Ekblom and Hermansen 1968) his maximal oxygen uptake was increased slightly to 5.60 l/min. This oxygen uptake was brought about by a cardiac output of 34.4 l/min and an arteriovenous oxygen difference of 163 ml per liter of blood. The hemoglobin concentration on this occasion was 14.7 g/100 ml and the cardiac output per liter of oxygen consumed was 6.1 liters. Other results which add circumstantial support to the concept that variations in the hemoglobin concentration might be compensated for by changing the cardiac output are reported by Grimby et al. (1966). In this study old (45–55 years) still active athletes were found to have an average maximal oxygen uptake of 3.56 l/min. The corresponding value for the cardiac output was 26.8 l/min. Hemoglobin concentration was 13.5 g/100 ml (average value). This gives an average cardiac output of 7.5 liters of blood per liter of oxygen consumed. In the study by Åstrand et al. (1964) in young healthy males the corresponding figure was 5.9 liters blood per liter of oxygen consumed. The mean value for the hemoglobin concentration was 15.6 g/100 ml in these subjects. Female subjects are known to have lower mean values for hemoglobin concentration. Consequently a higher cardiac output per liter of oxygen consumed should be anticipated both during submaximal and maximal work. In fact this was shown by Åstrand et al. (1964). The mean values for hemoglobin concentration and oxygen uptake during maximal work were 13.6 g/100 ml and 2.60 l/min, respectively. The corresponding mean value for cardiac output was 18.4 l/min, representing 7.1 liters of blood per liter of oxygen consumed. In another group of subjects with fairly low hemoglobin values, the pubertal boys studied by Eriksson (1972) a cardiac output of 6.9 liters per liter of oxygen consumed during maximal exercise was observed. In the anemic subjects studied by Sproule et al. (1960) the corresponding value was 12.8 liters of blood per liter of oxygen consumed. Thus, there seems to be considerable evidence which suggests that variations in the hemoglobin concentration could be compensated for by changing the cardiac output.

Another consideration that adds support to the concept of compensatory mechanisms for th capacity to transport oxygen in relationship to variations n the hemoglobin concentration is the series of studies showing an important relationship between the metabolism of the red blood cells and its function as an organ for oxygen transport (Chanutin and Curnish 1967. Benesch and Benesch 1967. de Verdier et al. 1969. Brewer and Eaton 1971). It was shown by Chanutin and Curnish (1967) and Benesch and Benesch (1967) that the presence of phosphorylated intermediates, 2,3-diphosphoglycerate (DPG) or adenosine triphosphate (ATP) caused a concomittant reduction in the oxygen affinity of hemoglobin solutions. Eaton and Brewer (1968). Eaton et al. (1969. 1970) showed a signif

negative correlation between red blood cells DPG and the hemoglobin concentration Experimentally produced polycythemia in rats is shown to cause low levels of DPG (Gerlach et al 1970) while in anemia of various etiologies the level of DPG is high (Hjelm 1969 Eaton et al 1970) The results of these studies indicate that for instance a lowering of the hemoglobin concentration might cause changes in the red blood cells by which more oxygen is released from the hemoglobin at a certain oxygen tension in the tissue Furthermore it is also shown that the rate of desaturation may be significantly increased in anemic subjects (Brewer and Eaton 1971) That these mechanisms probably play an important role for the oxygen transport during maximal exercise in subjects having low hemoglobin concentrations is indicated by the low oxygen content of femoral venous blood (i.e. 1.35 vol %) in the anemic subjects studied by Sproule et al (1960) compared with the corresponding value in normal subjects, i.e. 6.88 vol.% (Mitchell et al 1958a)

Although the results of the present study and those of many others indicate that variations in the hemoglobin concentration may be effectively compensated by changing the cardiac output and/or arteriovenous oxygen difference studies with physical models (Crowell and Smith 1967) and in dogs (Murray et al. 1962) have suggested that there seems to be an optimal hematocrit where the oxygen transport is maximal The studies of Murray et al. (1962) also indicated that the hemodynamic adjustments in normovolemic anemia and polycythemia were insufficient to maintain normal oxygen delivery

The capacity of the heart to increase the cardiac output is, maybe the factor most frequently considered as the main limiting factor (Mitchell et al 1958a Åstrand and Saltin 1961b Rowell 1962 Bevegård and Shepherd 1967 Ouellet et al 1969 Gollnick et al. 1972, Miyamura and Honda 1972 and others) The view cardiac output is an essential factor for the magnitude of the maximal oxygen uptake, is supported by evidence from studies on patients with pure mitral stenosis (Blackmon et al. 1967) sedentary healthy subjects (Åstrand et al 1964) and endurance athletes (Ekblom and Hermansen 1968) These studies showed that subjects with low maximal oxygen uptake had low cardiac outputs while subjects with high maximal oxygen uptakes were characterized by high cardiac outputs.

The cardiac output is determined by the product of heart rate and stroke volume. Accordingly cardiac output may be limited by an inability to increase the heart rate to that of the stroke volume. With regard to the first possibility it is worth noticing that the maximal heart rate is found to be approximately the same (190–200 beats/min) in subjects with low average and high maximal cardiac outputs (Åstrand et al. 1964 Blackmon et al. 1967 Ekblom and Hermansen 1968). Physical training increases both maximal oxygen uptake and maximal" cardiac output (Rowell 1962 Saltin et al. 1968 Ekblom 1969) Maximal heart rate, however remains almost unchanged. There are even studies indicating a lowering of the maximal heart rate in connection with training (Andersen 1967a, Davies 1967)

Changes in the stroke volume seem to be the most important factor by which cardiac output is altered in subjects with "normal" hemoglobin values (Åstrand et al. 1964 Blackmon et al. 1967 Ekblom and Hermansen 1968) This was also the case in the present investigation where a 6 percent higher cardiac output was observed during uphill running than during bicycling This increase in cardiac output was brought about by only a small (approximately 1 percent) increase in

maximal heart rate, while increased stroke volume contributed approximately 5 percent. Altered stroke volume seems also to be an important factor with regard to the observed changes in cardiac output with variations in the hemoglobin concentration (Ekblom and Hermansen 1968) Although the mechanisms by which the stroke volume is altered are unknown, results from animal studies and investigations on human subjects seem to indicate that cardiac and extracardiac factors are involved.

With regard to the changes in stroke volume (i.e cardiac output) in relation to variations in the hemoglobin in the hemoglobin concentration (i.e hematocrit) and to exercise (or a combination of both) the present study and other investigations (Murray et al. 1962, Fowler and Holmes 1971) seem to indicate that changes in the peripheral resistance may be one important factor The mechanisms by which the peripheral resistance is varied in the intact organisms are unclear but variations in the viscosity of the blood are one essential factor (Murray et al. 1962 Crowell and Smith 1967 Agarwal et al. 1970) However as indicated by Escobar et al (1966) and Fowler and Holmes (1971) improved ventricular function is another possibility The reflex control of the veins is another important factor with special reference to the changes during exercise (Bevegård and Shepherd 1965 Shepherd 1966). However if or to what extent the reflex control of the veins is influenced by the hematocrit is not known.

Although considerable evidence indicates that the maximal oxygen uptake is limited by the capacity of the heart to transport oxygen (i.e blood) to the working muscles, several investigators have suggested that maximal oxygen uptake is limited by peripheral factors. Åstrand (1952) for instance suggested that the capacity of the heart to increase the cardiac output is greater than the ability of the muscles to receive it. Thus, it was indicated that the limiting factor might be found in the capacity of the vascular bed of the muscles.

In the present study the capillary density was found to be approximately 600 capillaries per mm² both in the well-trained and untrained subjects. The maximal oxygen uptake, however was 71 4 ml/kg x min in the well-trained and 50.2 ml/kg x min in the untrained subjects. Thus, the well-trained subjects were able to transport approximately 20 ml oxygen per kilogram body weight and minute more than the untrained subjects. Although there might be a difference in the lean body mass between the well-trained and untrained subjects it still means that a larger amount of oxygen is transported per unit muscle weight and tim by the well-trained subjects. Thus, it appears that the number of capillaries per mm² as such does not represent a limiting factor for the transport of oxygen during maximal exercise in man. However it was also shown in the present investigation that the average size of the muscle cells was larger in the well-trained as compared to the untrained subjects. Consequently each muscle cell was supplied with a larger number of capillaries in subjects with high maximal oxygen uptake compared with subjects with lower maximal oxygen uptake. However when the diffusion distance was calculated according to the method of Krogh (1919) no statistically significant differences between the two groups of subjects could be observed. Thus, according to the results of the present study it appears that the diffusion distance as such, probably does not limit the oxygen transport in these subjects.

Mathematical models describing the oxygen transport in peripheral tissues 1

negative correlation between red blood cells DPG and the hemoglobin con tion. Experimentally produced polycythemia in rats is shown to cause low le DPG (Gerlach et al. 1970) while in anemia of various etiologies the level of high (Hjelm 1969 Eaton et al 1970) The results of these studies indicate th instance a lowering of the hemoglobin concentration might cause changes red blood cells by which more oxygen is released from the hemoglobin at a c oxygen tension in the tissue Furthermore it is also shown that the ra desaturation may be significantly increased in anemic subjects (Brewer and I 1971) That these mechanisms probably play an important role for the ox transport during maximal exercise in subjects having low hemoglobin conce tions is indicated by the low oxygen content of femoral venous blood (i.e vol %) in the anemic subjects studied by Sproule et al (1960) compared with corresponding value in normal subjects, i e 6 88 vol.% (Mitchell et al. 195

Although the results of the present study and those of many others indicate t variations in the hemoglobin concentration may be effectively compensated changing the cardiac output and/or arteriovenous oxygen difference, studies w physical models (Crowell and Smith 1967) and in dogs (Murray et al 1962) ha suggested that there seems to be an optimal hematocrit where the oxygen transpo is maximal. The studies of Murray et al (1962) also indicated that th hemodynamic adjustments in normovolemic anemia and polycythemia we insufficient to maintain normal oxygen delivery

The capacity of the heart to increase the cardiac output is, maybe the facto most frequently considered as the main limiting factor (Mitchell et al 1958a Åstrand and Saltin 1961b Rowell 1962 Bevegård and Shepherd 1967 Ouellet et 1969 Gollnick et al. 1972 Miyamura and Honda 1972 and others) The view cardiac output is an essential factor for the magnitude of the maximal oxygen uptake, is supported by evidence from studies on patients with pure mitral stenosis (Blackmon et al. 1967) sedentary healthy subjects (Åstrand et al 1964) and endurance athletes (Ekblom and Hermansen 1968) These studies showed that subjects with low maximal oxygen uptake had low cardiac outputs while subjects with high maximal oxygen uptakes were characterized by high cardiac outputs.

The cardiac output is determined by the product of heart rate and stroke volume. Accordingly cardiac output may be limited by an inability to increase the heart rate to that of the stroke volume. With regard to the first possibility it is worth noticing that the maximal heart rate is found to be approximately the same (190—200 beats/min) in subjects with low average and high maximal cardiac outputs (Åstrand et al. 1964 Blackmon et al 1967 Ekblom and Hermansen 1968) Physical training increases both maximal oxygen uptake and "maximal" cardiac output (Rowell 1962 Saltin et al 1968 Ekblom 1969) Maximal heart rate, however remains almost unchanged There are even studies indicating a lowering of the maximal heart rate in connection with training (Andersen 1967a, Davies 1967).

Changes in the stroke volume seem to be the most important factor by which cardiac output is altered in subjects with normal hemoglobin values (Åstrand et al. 1964 Blackmon et al. 1967 Ekblom and Hermansen 1968) This was also the case in the present investigation where a 6 percent higher cardiac output was observed during uphill running than during bicycling This increase in cardiac output was brought about by only a small (approximately 1 percent) increase in

proposed by Stainby and Otis (1964). However whether or not the oxygen tension of the venous blood is representative for the oxygen tension in all capillaries is unknown. It is, for instance unlikely that all motor units are activated at the work intensity necessary to obtain the maximal oxygen uptake. Consequently the venous blood draining the muscle will probably be a mixture of blood with low and higher oxygen tensions. Furthermore Forster (1967) has indicated that there are differences in the oxygen tensions, not only between the venous blood and the cells, but also within the muscle cell itself. There is also evidence which suggests that there might be a small oxygen difference in the oxygen tension even within the mitochondria (Forster 1967). If this is correct it seems difficult to assess the oxygen tension in the cells from measurements of oxygen tension in the venous blood from the tissue. Thus, a high oxygen tension in the venous blood does not necessarily mean that the oxidative capacity of the muscles represents the limiting factor.

With regard to the arteriovenous oxygen difference during maximal exercise surprisingly small differences have been observed between groups of subjects with large differences in maximal oxygen uptake. Thus, the arteriovenous oxygen difference was found to be approximately the same in patients with mitral stenosis (Blackmon et al. 1967) sedentary healthy subjects (Åstrand et al 1964) and athletes (Rowell 1962 Ekblom and Hermansen 1968). On the other hand it was shown by Rowell (1962) and Saltin et al (1968) that the arteriovenous oxygen difference was increased after training. Holloszy and co-workers (Holloszy 1967 Holloszy et al. 1971) have shown that there is an increase in the mitochondrial enzymes during physical training. The higher oxygen uptake obtained after training would according to Holloszy et al. (1971) be explained by the higher capacity to oxidize pyruvate. However in the results of Gollnick et al (1972) it was indicated that the oxidative capacity of the skeletal muscles of both trained and untrained subjects exceeded the values observed for maximal oxygen uptake.

Thus, although the present investigation has provided evidence which may be interpreted to support the view that maximal oxygen uptake is limited by the capacity of the circulatory system to transport oxygen to the muscles, there are still several questions yet to be answered

been worked out by Krogh (1919) Fenn (1927) Hill (1928) Roughton (1952) Kety (1957) Hill (1965) and Thews (1968) Although there seems to be general agreement about the diffusion theory necessary experimental evidence to verify the calculations in the different models has been difficult to obtain. According to the above cited studies a high number of capillaries would facilitate the oxygen transport in the muscles. However the result of the present investigation showed that the well-trained subjects were able to transport more oxygen per unit muscle mass and time than the untrained subjects, the diffusion distance being the same. However with regard to the oxygen diffusion in skeletal muscles, the capillary density is probably only one of the many important factors. The presence of myoglobin is another important factor It was shown by Scholander and Hemmingsen (Scholander 1960 Hemmingsen and Scholander 1960 Hemmingsen 1963 Hemmingsen 1965) that the presence of hemoglobin or myoglobin in a solution may facilitate the oxygen diffusion It has also been suggested that the presence of myoglobin in the skeletal muscle may enhance the oxygen diffusion and increase the oxygen supply to a significant extent According to the calculations of Wittenberg (1965) the flux of oxygen facilitated by myoglobin is about 6 times greater than the ordinary diffusive flux. In other words, approximately 80 percent of the oxygen concerned reaches the mitochondria by myoglobin facilitated diffusion It was shown by Scholander (1960) that the oxygen flux increased with increasing concentration of the pigment (myoglobin or hemoglobin) As indicated by the studies of Whipple (1926) on dogs, and by Pettengale and Holloszy (1967) on rats, physical training may increase the concentration of myoglobin in skeletal muscle The results of Gollnick et al (1972) might indicate that this also happens in human skeletal muscle during training Thus, if this concept is correct the higher oxygen uptake observed in the well-trained subjects of the present investigation may be at least partly explained by a higher concentration of myoglobin in the muscles.

The ability of the active tissues to extract oxygen delivered by the cardiovascular system is another possible limiting factor If this was the case, a low arteriovenous oxygen difference should be expected. A low arteriovenous oxygen difference could be due to an inefficient distribution of the cardiac output In fact, this was observed in patients with vasoregulatory asthenia (Holmgren et al 1957) A low arteriovenous oxygen difference could however also be due to a metabolic deficiency i.e an inability of the mitochondria to oxidize pyruvate. This possibility is supported by the results of Kaijser (1970) who reported only moderately reduced blood lactate concentration in deep venous blood although the arterial oxygen tension was increased significantly The author concluded that the links in the oxygen transport system must be fully saturated with oxygen and that the limiting factor for the oxygen consumption must be found at the mitochondrial level. Results from measurements of the oxygen tension in the venous blood draining the muscles during maximal exercise (Keul et al 1967 Doll et al 1968 Kaijser 1970, Pirnay et al. 1972) are also interpreted to support the view that oxygen consumption is limited by the capacity of the muscles to consume the oxygen brought to them In all these studies, the oxygen tension in the venous blood was found to be higher than the critical oxygen tension, (i.e the oxygen tension below which the rate of oxygen uptake by the muscles is unaffected) of 10 mm Hg as

increased markedly while maximal oxygen uptake remained at the same level It is emphasized, however that severely anemic subjects were not included in the study and further examinations of such subjects are necessary to ascertain the limit below which anemia causes measurable impairment of the capacity to transport oxygen

Capillary density in muscle samples obtained by needle biopsies from the lateral portion of the muscle quadriceps femoris was measured using standard histological techniques and the PAS-staining reaction in 8 untrained students and 7 well trained endurance athletes. Mean values (± SE) of the capillary density were found to be 611 ± 54.5 and 599 ± 26.6 for the well trained and the untrained subjects, respectively Statistical analysis revealed no significant difference between the two groups of subjects. The average size of the muscle fibres in the well-trained muscle was found to be significantly larger than in the untrained muscle. Consequently the number of capillaries per muscle fibre was higher in the well-trained subjects than in the untrained ones. Calculations of the diffusion distance (i.e. average half-distance between two capillaries) in the well-trained and untrained muscle, revealed no statistically significant difference

The variations in maximal oxygen uptake in relation to sex age and level of physical activity in different groups of the Norwegian population were investigated Altogether 593 female and male subjects between 11 and 59 years of age were studied. The results obtained in the different groups of the Norwegian population are compared with similar results from other countries.

On the whole, the mean values for maximal oxygen uptake in the Norwegian subjects were higher than in all other studies reported in the literature

REFERENCES

AGARWAL, J.B R. PALTOO and W H PALMER, Relative viscosity of blood at varying hematocrits in pulmonary circulation. *J appl. Physiol.* 1970. 29 866–871

ANDERSEN H T and H. BARKVE Iron deficiency and muscular work performance. *Scand. J clin. Lab Invest.* 1970 25 Suppl. 114

ANDERSEN K.L., The effect of physical training with and without cold exposure upon physiological indices of fitness for work. *Canad. Med. Ass. J* 1967a. 96. 801–803

ANDERSEN K.L., Ethnic group differences in fitness for sustained and strenuous muscular exercise *Canad. Med. Ass. J* 1967b 96 83*–833

ANDERSEN K.L., R.W ELSNER, B SALTIN and L. HERMANSEN Physical fitness in terms of maximal oxygen intake of nomadic Lapps. *Technical report. Institut of Work Physiology* Oslo 1961

ANDERSEN K.L. and L. HERMANSEN Aerobic work capacity in middle-aged Norwegian men. *J appl. Physiol.* 1965 20 43 –436.

ASMUSSEN E. and M. NIELSEN Studies on the regulation of respiration in heavy work. *Acta physiol. scand.* 1946 12 171–188

ASMUSSEN E and L HEMMINGSEN Determination of maximum working capacity at different ages in work with the legs or with the arms. *Scand. J clin. Lab Invest.* 1958. 10. 67–71

ASMUSSEN E and M. NIELSEN Pulmonary ventilation and effect of oxygen breathing in heavy exercise *Acta physiol. scand.* 1958 43 365–378.

ASMUSSEN E. and M. NIELSEN Alveolo-arterial gas exchange at rest and during work at different O_2 tensions. *Acta physiol. scand.* 1960 50 153–166

ÅSTRAND I. Aerobic work capacity in men and women with special reference to age. *Acta physiol. scand.* 1960 49 Suppl. 169

ÅSTRAND P.-O., Experimental studies of physical working capacity in relation to sex and age Munksgaard. Copenhagen. 1952.

ÅSTRAND P.-O Aerobic work capacity during maximal performance under various conditions. *Circulation Res.* 1967 20 and 21 202–210

ÅSTRAND P.-O and I RYHMING A nomogram for calculation of aerobic capacity (physical fitness) from pulse rate during submaximal work. *J appl. Physiol.* 1954 7 218–221

ÅSTRAND P.-O and B SALTIN Oxygen uptake during the first minutes of heavy muscular exercise *J appl. Physiol.* 1961a. 16 971–976.

ÅSTRAND P.-O and B. SALTIN Maximal oxygen uptake and heart rate in various types of muscular activity *J appl. Physiol.* 1961b 16. 977–981

ÅSTRAND P.-O T.E. CUDDY B SALTIN and J STENBERG Cardiac output during submaximal and maximal work. *J appl. Physiol.* 1964 19 268–274.

BALKE, B G.P GRILLO E.B KONECCI and U C. LUFT Work capacity after blood donation. *J appl. Physiol.* 1954 7 231—238

BARNARD R.J., V.R. EDGERTON T FURUKAWA and J B PETER Histochemical, biochemical, and contractile properties of red white and intermediate fibers. *Amer J Physiol.* 1971 2?0 410—414

BENESCH R. and R.E. BENESCH The effect of organic phosphates from the human erythrocytes on the allosteric properties of hemoglobin. *Biochem. Biophys. Res. Commun.* 1967 ?6 16?—167

BERGSTRÖM, J Muscle electrolytes in man. *Scand. J clin. Lab Invest.* 1962 14 Suppl. 68

BEUTLER, E., S. LARSH and F TANZI Iron enzymes in iron deficiency VII Oxygen consumption measurements in iron-deficient subjects. *Amer J Med. Sci.* 1960 239 759—765

BEVEGÅRD B.S and J T SHEPHERD Changes in tone of limb veins during supine exercise. *J appl. Physiol.* 1965 20 1—8.

BEVEGÅRD B.S and J T SHEPHERD Regulation of the circulation during exercise in man. *Physiol. Rev* 1967 47 178—?13

BINKHORST R.A. J POOL, P van LEEUWEN and A BOUHUYS Maximal xygen uptake in healthy nonathletic males. *Int. Z. angew Physiol.* 1966. 10—18.

BLACKMON J R., L.B. ROWELL, J W KENNEDY R.D TWISS and R.D CONN Physiological significance of maximal oxygen intake in "pure" mitral stenosis. *Circulation* 1967 36. 497—510

BOLINDER, E., E. MAGNUSSON and L. NYREN LO-Medlemmarnas subjektiva uppfatning om arbetsplatsens hälsorisker *Bokförlag 4 Landsorganisationen i Sverig* Stockholm. 1969

BREWER, G.J and J W EATON Erythrocyte metabolism Interaction with oxygen transport. *Science* 1971 171 1205—1211

BURKE R.E., D.N LEVINE and F E. ZAJAC, III., Mammalian motor units Physiological-histochemical correlation in three types in cat gastroenemius. *Science* 1971 174. 709—712

BUSKIRK, E. and H.L. TAYLOR, Maximal oxygen intake and its relation to body composition, with special reference to chronic physical activity and obesity *J appl. Physiol.* 1957 11 72—78.

CHANUTIN A. and R.R. CURNISH Effect of organic and inorganic phosphorates on the oxygen equilibrium of human erythrocytes. *Arch. Biochem. Biophys.* 1967 121 96—102

CHASE, G.A. C. GRAVE and L.B ROWELL, Independence of changes in functi nal and performance capacities attending prolonged bed rest. *Aerospa Med.* 1966. 37 1232—1?38

CHRISTENSEN E.H., Beiträge zur Physiologie schwerer körperlicher Arbeit II Mitteilung Die Korpertemperatur während und unmittelbar nach schwerer körperlicher Arbeit. *Arbeitsphysiol.* 1931 4 154—174.

CHRISTENSEN E.H. Beiträge ur Physiologie schwerer korperlicher Arbeit. VI Mitteilung Der Stoffwechsel und die respiratorischen Funktionen bei schwerer körperlicher Arbeit. *Arbeitsphysiol.* 1932. 5 463—478

CHRISTENSEN E.H., A. KROGH and J LINDHARD Recherches sur l effort musculaire intense Bulletin trimestriel d l organisation d'hygiène *Société des Nations.* 1934 3 407—439

CHRISTENSEN E.H. and P HÖGBERG Physiology of skiing *Arbeitsphysiol.* 1950. 14. 292—303

COHN J E. D G CARROLL, B W ARMSTRONG R.H. SHEPARD and R.L. RILEY Maximal diffusing capacity of the lung in normal male subjects of different ages. *J appl. Physiol.* 1954 6 588—597
COTES J.E. J.M DABBS P C. ELWOOD A.M HALL, A. McDONALD and M.J SAUNDERS The response to submaximal exercise in adult females; relation to haemoglobin concentration. *J Physiol.* (Lond.) 1969 203 79—80.
CROWELL J W and E.E. SMITH Determinant of the optimal hematocrit. *J appl. Physiol.* 1967 22 501—504
CUMMING R.G Current levels of fitness. *Canad. Med. Ass. J* 1967 96 868—877
DAVIES C.T.M Commentary *Canad. Med. Ass. J* 1967 96. 743—744
DAVIES C.T.M., The oxygen-transporting system in relation to age. *Clin. Sci.* 1972. 42. 1—13
DAVIES C.T.M. C. BARNES R H. FOX, R.O OJIKUTU and A.S SAMUELOFF Ethnic differences in physical working capacity *J appl. Physiol.* 197 33 7*6—732.
de VERDIER, C. H. L. GARBY and M. HJELM The role of 2,3-diphosphoglycerate in the erythrocyte as a regulator of tissue oxygen tension. *Försvarsmedicin* 1969 5 258—259
DEHN M.M and R.A. BRUCE, Longitudinal variations in maximal oxygen intake with age and activity *J appl. Physiol.* 197? 33 805—807
DOLL, E., J KEUL and C. MAIWALD Oxygen tension and acid-base equilibria in venous blood of working muscle *Amer J Physiol.* 1968. ?15 23—29
DRIPPS R.D and J.H. COMROE The respiratory and circulatory response of normal men to inhalation of 7 6 and 10 4% CO_2 with a comparison of the maximal ventilation produced by severe muscular exercise inhalation of CO_2 and maximal hyperventilation *Amer J Physiol.* 1947 149 43—51
[illegible] B. and A.G.E. PEARSE A comparative histochemical study of oxidative enzymes and phosphorylase activity in skeletal muscle. *Histochemie* 1960 2 105—117
DÖBELN W v., A simple bicycle ergometer *J appl. Physiol.* 1954 7 22?—?24
DÖBELN W Maximal oxygen intake, body size and total hemoglobin in normal man *Acta physiol. scand.* 1956. 38. 193—199
EATON J W and G J BREWER, The relationship between red cell 2,3-diphosphoglycerat and levels of hemoglobin in the human. *Proc. U S. Nat. Acad. Sci.* 1968 61 756—760
EATON J W., G.J BREWER and R.F GROVER Role of red cell ,3 diphosphoglycerate in the adaption of man to altitude. *J Lab clin. Med.* 1969 73 603—609
EATON J W., G.J BREWER, J.S SCHULTZ and C.F SING Variations in 2,3-diphosphoglycerate and ATP levels in human erythrocytes and effects on oxygen transport. In Red cell metabolism and function. ed. G.J Brewer Plenum Press. New Yo k. 1970
EDSTRÖM, L. and B NYSTRÖM Histochemical types and sizes of fibres of normal human muscles. *Acta neurol. scand.* 1969 45 257—269
EDSTRÖM, L. and B EKBLOM Differences in size of red and white muscle fibres in vastus lateralis of musculus quadriceps femoris of normal individuals and athletes. Relation to physical performance *Scand. J clin. Lab Invest.* 1972. 30 175—181
EKBLOM, B Effect of physical training on oxygen transport system in man. *Acta physi l. scand.* 1969 Suppl 3 8

EKBLOM B., P-O ASTRAND B SALTIN J STENBERG and B WALLSTRÖM Effect of training on circulatory response to exercise *J appl. Physiol.* 1968. 24 518–528.
EKBLOM, B and E. GJESSING Maximal oxygen uptake of the Easter Island population. *J appl. Physiol.* 1968. 25 124–129
EKBLOM, B. and L. HERMANSEN Cardiac output in athletes. *J appl. Physiol.* 1968. 25 619–625
EKBLOM, B., A.N GOLDBARG and B GULLRING Response to exercise after blood loss and reinfusion. *J appl. Physiol.* 1972 33 175–180
ERICSSON P The effect of iron supplementation on the physical work capacity in the elderly *Acta med. Scand.* 1970 188 361–374
ERIKSSON B.O., Physical training oxygen supply and muscle metabolism in 11–13 year old boys *Acta physiol. scand.* 197 Suppl 384
ESCOBAR, E., N.L. JONES E RAPAPORT and J F MURRAY Ventricular performance in acute normovolemic anemia and effects of beta blockade *Amer J Physiol.* 1966 211 877–884
FAULKNER, J.A., D.E. ROBERTS R.E. ELK and J CONWAY Cardiovascular responses to submaximum and maximum effort cycling and running *J appl. Physiol.* 1971 30 457–461
FENN W.O The oxygen consumption of nerve during stimulation. *J gen. Physiol.* 1927 10 767–779
FOLKOW B., P GASKELL and B.A. WAALER Blood flow through limb muscles during heavy rhythmic exercise. *Acta phy iol. scand.* 1970 80 61–72.
FOLKOW B., U HAGLUND M. JODAL and O LUNDGREN Blood flow in the calf muscle of man during heavy rhythmic exercise. *Acta physi l. scand.* 1971 81 157–163
FORSTER, R.E., Oxygenation of the muscle cell. *Circulation Res.* 1967 20 and 1 115–121
FOWLER, N.O and J C. HOLMES Ventricular function in anemia. *J appl. Physiol.* 1971 31 260–265
FREEDMAN S., Sustained maximal voluntary ventilation. *Resp. Physiol.* 1970. 8. 230–244
GERLACH, E. J DUHM and B DEUTICKE Metabolism of 2,3-diphosphoglycerate in red blood cells under various experimental conditions. I Red Cell Metabolism and Function. ed. G. Brewer Plenum Press. New York. 1970 155–174
GLASSFORD R.G G.H.Y BAYCROFT A.W SEDGWICK and R.B J MACNAB Comparison of maximal oxygen uptake values determined by predicted and actual methods. *J ppl. Phy iol.* 1965 20 509–513
GOLLNICK, P.D R.B. ARMSTRONG C.W SAUBERT IV K. PIEHL and B. SALTIN Enzyme activity and fiber composition in skeletal muscle of untrained and trained men. *J appl. Physiol.* 1972 33 312–319
GRIMBY G N.J NILSSON and B. SALTIN Cardiac output during submaximal and maximal exercise in active middle-aged thletes. *J appl. Physiol.* 1966. 21 1150–1156.
GRIMBY G., E. HAGGENDAL and B. SALTIN Local xenon 133 clearance from the quadriceps muscl during exercise in man. *J appl. Physiol.* 1967 22 305–310
GRIMBY G., L. WILHELMSEN B EKSTRÖM-JODAL, M. AURELL, J J P and G TIBBLIN Aerobi power and related factors in a population men aged 54 *Scand. J clin. Lab. Invest.* 1970 26. 287–294

HAMMERSEN F The pattern of the terminal vascular bed and the ultrastructure of capillaries in skeletal muscle. In Oxygen transport in blood and tissue. eds. D W Lübbers, U C. Luft, G Thews and E. Witzleb Georg Thieme Verlag. Stuttgart 1968. 184–197
HECHT A., Zur Capillären Gefässversorgung der subendokardialen Muskelschichten in menschlichen Herzen. *Arch. Pathol. Anat.* 1958 331 26–52
HEMMINGSEN E.A., Enhancement of oxygen transport by myoglobin. *J comp. Biochem. Physiol.* 1963 10 239–244
HEMMINGSEN E.A. Transfer of oxygen through solutions of hemi pigments. *Acta physiol. scand.* 1965 Suppl. 246
HEMMINGSEN E. and P F SCHOLANDER Specific transport of oxygen through hemoglobin solutions. *Science* 1960 132 1379–1381
HENNEMAN E. G SOMJEN and D O CARPENTER Functional significance of cell size in spinal motoneurons. *J Neurophysiol.* 1965 28 560–580
HERMANSEN L. Aerob arbeidskapasitet i relasjon til alder og kjønn. *Cand. real. thesis,* Institute of Zoophysiology University of Oslo Norway 1964
HERMANSEN L. and K.L. ANDERSEN Aerobic work capacity in young Norwegian men and women. *J appl. Physiol.* 1965 20 425–431
HERMANSEN L. and B EKBLOM Physical fitness of an arctic and a tropical population. In Physical activity in health and disease. eds. K. Evang and K. Lange Andersen Universitetsforlaget Oslo 1966 231–234
HERMANSEN L. and H.B SKASET Unpublished results. 1970
HERMANSEN L. and S. OSEID Unpublished results. 1971a.
HERMANSEN L. and S. OSEID Direct and indirect estimation of maximal oxygen uptake in pre-pubertal boys. *Acta paediat. scand.* 1971b Suppl. 217 18–23
.. GER, T., N C. BIRKHEAD S.M HORVATH B ISSEKUTZ Jr and K. ODAHL Assessment of physical work capacity *J appl. Physiol.* 1961 16. 153–159
HILL, A.V The role of oxidation in maintaining the dynamic equilibrium of the muscle cell. *Proc Roy Soc.* London. Ser B. 1928 103 138–162
HILL, A.V Trails and trials in physiology Arnold. London. 1965
HILL, A.V and H LUPTON Muscular exercise, lactic acid and the supply and utilization of oxygen. *Quart. J Med.* 1923 16 135–171
HILL A.V., C.N.H. LONG and H. LUPTON Muscular exercise lactic acid, and the supply and utilization of oxygen. VII and VIII Muscular exercise and oxygen uptake *Proc. Roy Soc* London Ser B 1924 97 155–176.
HJELM M., The content of 2,3-diphosphoglycerate and some other phosphocompounds in human erythrocytes from healthy adults and subjects with different types of anemia. *Försvarsmedicin.* 1969 5 219–226
HOES M.J.A.J.M., R.A. BINKHORST A.E.M.C. SMEEKES-KUYL and A.C.A. VISSERS Measurements of forces exerted on pedal and crank during work on a bicycle ergometer at different loads. *Int. Z. angew Physiol.* 1968. 26. 33–42
HOLLOSZY J O Biochemical adaptation in muscle Effects of exercise on mitochondrial oxygen uptake and respiratory enzyme activity in skeletal muscle. *J biol. Chem.* 1967 242 2278–2 82
HOLLOSZY J O L.B OSCAI P.A. MOLÉ and I.J DON Biochemical adaptations to endurance exercise in skeletal muscle. In Muscle metabolism during exercise. eds. B Pernow and B Saltin. Plenum Press. New York. 1971 51–61
HOLMÉR, I. Oxygen uptake during swimming in man. *J ppl. Physiol.* 1972 33 502–509
HOLMGREN A. Cardiorespiratory determinants of cardiovascular fitness. *Canad. Med. Ass. J* 1967 96. 697–702

HOLMGREN A., B JONSSON M. LEVANDER H. LINDERHOLM T SJÖSTRAND and G STRÖM, Low physical working capacity in suspected heart cases due to inadequate adjustment of peripheral blood flow (vasoregulatory asthenia) *Acta med. Scand.* 1957 158 413—436.
HOLMGREN A. and H. LINDERHOLM, Oxygen and carbon dioxide tensions of arterial blood during heavy and exhaustive exercise *Acta physiol. scand.* 19 8 44. 203—215
HOLMGREN A. and B PERNOW Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output A comparison with the van Slyke method. *Scand. J clin. Lab Invest.* 1959 11 143—149
HOLMGREN A. and P-O ÅSTRAND D_L and the dimensions and functional capacities of the O_2 transport system in humans. *J appl. Physiol.* 1966. 21 1463—1470.
HORT W., Capillarisation of the myocardium under normal and pathological condition. In Oxygen transport in blood and tissue eds. D W Lübbers U C. Luft, G Thews and E. Witzleb. Georg Thieme Verlag. Stuttgart. 1968. 150—158
IKAI M., M. SHINDO and M. MIYAMURA, Aerobic work capacity of Japanese people. *Res. J Phys. Ed* 1970. 14 137—142
IKAI, M. and K. KITAGAWA, Maximum oxygen uptake of Japanese related to sex and age. *Medicine and Science in Sports.* 1972 4 127—131
JORFELDT L. Metabolism of L (+)— lactate in human skeletal muscle during exercise. *Acta physiol. scand.* 1970 78. Suppl. 338
KAIJSER, L., Limiting factors for aerobic muscle performance *Acta physiol. scand.* 1970. Suppl. 346.
KAMON E. and K.B PANDOLF Maximal aerobic power during laddermill climbing uphill running and cycling. *J appl. Physiol.* 1972. 32 467—473
KARLSEN J E. Arbeidsmiljø og arbeidsskader En undersøkelse blant LO s medlemmer om helserisikoene på arbeidsplassene. *Landsorganisasjonen i Norg* Informasjonskontoret. Oslo 1972.
KARLSSON J Lactate and phosphagen concentrations in working muscle in man. *Acta physiol. scand.* 1971 Suppl. 358.
KARPOVICH P V and N MILLMAN Athletes as blood donors. *Res. Quart.* 194 13 166—168.
KETY S.S., Determination of tissue oxygen tension. *Fed. Proc* 1957 16. 666—670
KEUL J., D KEPLER and E. DOLL, Lactate-pyruvate ratio and its relation to oxygen pressure in arterial coronary venous and femoral venous blood. *Arch. Intern. Physiol. Biochem.* 1967 75 573—578
KJELLBERG S R., U RUDHE and T SJÖSTRAND Increase of the amount f hemoglobin and blood volume in connection with physical training *Acta physiol. scand.* 1949a. 19 146—151
KJELLBERG S.R. U RUDHE and T SJÖSTRAND The amount of hemoglobin (blood volum) in relation to the pulse rate and heart volume during work. *Acta physiol. scand.* 1949b 19 152—168.
KLISSOURAS V., Heritability of daptive variation. *J appl. Physiol.* 1971 31 338—344
KNEHR, C.A. D B DILL and W NEUFELD Training and its effects on man at rest and at work. *Amer J Physiol.* 1942. 136. 148—156
KNUTTGEN H.G., Aerobic capacity of adolescents. *J appl. Physiol.* 1967 22 655—658.

KROGH A. The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue *J Physiol. (Lond.)* 1919 52 409–415
KROGH A., The anatomy and physiology of capillaries (revised ed.) Yale Univ Press. New Haven. 19?9
LARSEN Ö., Hemoglobin concentration hematocrit and MCHC in 19-year-old men. *Acta med. Scand.* 1966 180 6?1–630
LANDIS E.M. and J R. PAPPENHEIMER Exchange of substances through the capillary walls. In Handbook of Physiology *Circulation. Amer Physiol. Soc.* 1963 sect. 2. vol. II Chapt. ?9 961–1034
LUFT U C. D CARDUS T P.K. LIM E.C. ANDERSON and J L. HOWARTH Physical performance in relation to body size and composition *Ann. N Y Acad. Sci.* 1963 110 795–808.
MARGARIA, R., P CERRETELLI S MARCHI and L. ROSSI Maximum exercise in oxygen *Int. Z angew Physiol.* 1961 18 465–467
MARGARIA, R. P CERRETELLI P AGHEMO and G SASSI Energy cost of running. *J appl. Physiol.* 1963 18. 367–370
MITCHELL, J.H., B.J SPROULE and C.B CHAPMAN The physiological meaning of the maximal oxygen intake test *J Clin. Invest.* 1958a. 37 538–547
MITCHELL, J.H. B.J SPROULE and C.B CHAPMAN Factors influencing respiration during heavy exercise. *J Clin. Invest.* 1958b 37 1693–1701
MIYAMURA M and Y HONDA Oxygen intake and cardiac output during maximal treadmill and bicycle exercise *J appl. Physiol.* 197.. 32. 185–188.
MORRIS C.J., Human muscle fibre type grouping and collateral re-innervation. *J Neurol. Neurosurg. Psychiat.* 1968 32 440–444
AY J F., P GOLD and B L. JOHNSON Jr Systematic oxygen transport in normovolemic anemia and polycythemia. *Amer J Physiol.* 1962. 203 7 4
VIG H O.D VELLAR and J ANDERSEN Hemoglobin, hematocrit and values among boys and girls aged 7–?0 years in elementary and grammar schools. *Acta med. Scand.* 1967 182 183–191
NIELSEN M. and E. FRIDRICHSEN Eine Modifikation des Kroghschen Fahrrader gometers. *Skand. Arch. Physiol.* 1938 78. 76–82.
OUELLET Y S.C. POH and M.R. BECKLAKE, Circulatory factors limiting aerobic exercise capacity *J appl. Physiol.* 1969 ?7 874–880
PETRÉN T., T SJÖSTRAND and B SYLVÉN Der Einfluss des Trainings auf die Häufigkeit der Capillaren in Herz und Skelettmuskulatur *Arbeitsphysiol.* 1937 9 376–386.
PETTENGALE P K. and J O HOLLOSZY Augmentation of skeletal muscle myoglobin by a program of treadmill running *Amer J Physiol.* 1967 213 783–785
PIRNAY F M. LAMY J DUJARDIN R. DEROANNE and J.M. PETIT Analysis of femoral venous blood during maximum muscular exercise. *J appl. Physiol.* 1972 33 289–292.
PRUETT E.D R., Fat and carbohydrate metabolism in exercise and recovery and its dependence upon work load severity *Institut of Work Physiology.* Oslo. 1971
ROBBE H., Total amount of haemoglobin and physical working cap city in anaemia of pregnancy *Acta ob t. Gy ec. Scand.* 1958. 37 312–347
ROBINSON B F., S.E. EPSTEIN R.L. KAHLER and E. BRAUNWALD Circulatory effects of acute expansion of blood volume. *Circulation Res.* 1966. 19 26–32.

ROBINSON S., Experimental studies of physical fitness in relation to age *Arbeitsphysiol.* 1938 10. 251–373
ROBINSON S. and P.M. HARMON The effect of training and of gelatin upon certain factors which limit muscular work. *Amer J Physiol.* 1941 133 161–169
ROBINSON S., D B DILL, P.M HARMON F C. HALL and J W WILSON Adaptation to exercise of negro and white sharecroppers in comparison with Northern whites. *Human Biol.* 1941 13 139–158
RODAHL, K. P.-O ÅSTRAND N C. BIRKHEAD T HETTINGER B ISSEKUTZ Jr., D.M. JONES and R. WEAVER Physical work capacity A study of some children and young adults in the United States. *Arch. Environ. H alth.* 1961 2 499–510.
ROUGHTON F J W., Diffusion and chemical reaction velocity in cylindrical and spherical systems of physiological interest *Proc Roy Soc.* London Ser B 1952 140. 203–229
ROWELL, L.B., Factors affecting the prediction of maximal oxygen intake from measurements made during submaximal work with observations related to factors which may limit maximal oxygen uptake *Ph. D. thesis.* University of Minnesota. 196_.
ROWELL, L.B., Commentary In Proceedings of the International symposium on physical activity and cardiovascular health. *Canad. Med. Ass. J* 1967 96 735
ROWELL, L.B., H.L. TAYLOR and Y WANG Limitations to prediction of maximal oxygen intake *J appl. Physiol.* 1964 19 919–927
SALTIN B., Circulatory response to submaximal and maximal exercise after thermal dehydration. *J appl. Physiol.* 1964 19 1125–1132
SALTIN B and P-O ÅSTRAND Maximal oxygen uptake in athletes. *J appl. Physiol.* 1967 23 353–358.
SALTIN B G BLOMQVIST J.H. MITCHELL, R.L. JOHNSON K. WILDENTHAL, Jr and C.B. CHAPMAN Response to exercise after bed rest and after training. *Circulation.* 1968. 37 and 38. Suppl. 7
SCHOLANDER, P F., Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J biol. Chem.* 1947 167 235–250
SCHOLANDER P F Oxygen transport through hemoglobin solutions. *Science* 1960. 131 585–590.
SCHOLZ, R., H. SCHMITZ, T BUECHER and J.O LAMPEN Über die Wirkung von Nystatin auf Bäckerhefe. *Biochem. Z.* 1959 331 71–86.
SHEPHERD J T., Rol of the veins in the circulation. *Circulation.* 1966 33 484–491
SJÖSTRAND T., Volume and distribution of blood and their significance in regulating the circulation. *Physiol. Rev.* 1953 33 202–228
SPROULE B.J., J.H. MITCHELL and W F MILLER Cardiopulmonary physiological responses to heavy exercise in patients with anemia. *J Clin. Invest.* 1960. 39 378–388.
SPRYNAROVA, S Development of the relationship between aerobic capacity and th circulatory and respiratory reaction to moderate activity in boys 11–13 years old. *Physiol. bohemoslov.* 1966 15 253–264
STAINSBY W.N and A.B OTIS, Blood flow blood oxygen tension, oxygen uptake and oxygen transport in skeletal muscle. *Amer J Physiol.* 1964 206. 858–866.
STENBERG J., The significance of the entral circulation for the aerobic work capacity under various conditions in y ung healthy persons. *Acta physiol. scand.* 68. 1966. Suppl. 273

STENBERG J P-O ÅSTRAND B EKBLOM J ROYCE and B SALTIN Hemodynamic response to work with different muscle groups sitting and supine *J appl. Physiol.* 1967 2? 61–70

STRÖM, G The influence of anoxia on lactate utilization in man after prolonged muscular work. *Acta physiol. scand.* 1949 17 440–451

TAYLOR, H.L. E. BUSKIRK and A. HENSCHEL, Maximal oxygen intake as an objective measure of cardio-respiratory performance *J appl. Physiol.* 1955 8 73–80

THEWS G The theory of oxygen transport and its application to gaseous exchange in the lung In Oxygen transport in blood and tissue. Eds. D W Lübbers, U C. Luft G Thews and E. Witzleb Georg Thieme Verlag Stuttgart 1968. 1–?0

VANNOTTI A. and H PFISTER Untersuchungen zum Studium des Trainiertseins. IV Die Blutversorgung des ruhenden Muskels am trainierten Tiere. *Arbeitsphysiol.* 1933 7 127–133

VANNOTTI A. and M. MAGIDAY Untersuchungen zum Studium des Trainiertseins. V Über die Capillarisierung der trainierten Muskulatur *Arbeitsphysiol.* 1934 7 615–6?2.

WAALER H.T., Personal communication. 197

WEST J.B and C.T DOLLERY Distribution of blood flow and ventilation-perfusion ratio in the lung measured with radioactive CO_2 *J appl. Physiol.* 1960 15 405–410

WHIPPLE, G.H The hemoglobin of striated muscle. I Variations due to age and exercise. *Amer J Physiol.* 1926. 76. 693–707

WILLIAMS J.A. and J FINE Measurement of blood volume with a new apparatus. *New Engl. J Med.* 1961 264 842–846

?TTENBERG J.B., Myoglobin-facilitated diffusion of oxygen. *J gen. Physiol.* ?965 49 57–74

. AM C.B., N B STRYDOM, W F LEARY and C.G WILLIAMS Studies on the maximum capacity of men for physical effort. *Arbeitsphysiol.* 1966. 22 ?85–295

WYNDHAM, C.H N B STRYDOM, A.J van RENSBURG and G G ROGERS Effects on maximal oxygen intake of acute changes in altitude in a deep mine *J appl. Physiol.* 1970 ?9 552–555

Appendix

Table 1. Mean values for oxygen uptake, pulmonary ventilation, heart rate, blood lactate concentration and work time during maximal bicycle and treadmill exercise for groups A through G.

Subjects		Oxygen uptake l/min STPD		Pulmonary ventilation l/min BTPS		Heart rate beats/min		Blood lactate concentration mM		Work time minutes	
Groups	No.	Bicycle	Treadmill	Bicycle	Treadmill	Bicycle	Treadmill	Bicycle	Treadmill	Bicycle	Treadmill
A	8	4.63	5.04	151.8	161.5	188.1	192.3	13.0	13.4	5.6	5.6
		$p < 0.001$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
B	5	3.93	4.29	137 7	139 6	193.8	200.4	13.9	16.4	5.9	4 7
		$p < 0.001$		$p > 0.05$		$p < 0.05$		$p > 0.05$		$p < 0.05$	
C	6	3.17	3.54	130.2	132.9	196.0	197 8	16.4	17.6	4 7	4 6
		$p < 0.001$		$p > 0.05$		$p < 0.05$		$p > 0.05$		$p > 0.05$	
D	5	4.34	4.65	136.9	149.3	187.0	187 0	14.5	13.5	5.2	5.2
		$p < 0.001$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
E	10	4.55	4.75	136.5	134.5	178.6	179 7	13 4	14.3	4 4	5 0
		$p < 0.01$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
F	14	3.44	3.68	115 0	114.6	177.6	176.0	10.5	11.5	4 4	4.6
		$p < 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	
G	7	3.36	3.38	120.7	117.9	186.1	185.6	13.8	12.9	5.0	4.3
		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$		$p > 0.05$	

Table 2. Individual and mean values ± standard error f the mean and standard deviation for oxygen uptak ardiac output, heart rate stroke volume, arteriovenous oxygen difference, mean arterial pressure and arterial oxygen c tent during maximal bicycle and treadmill exercise

Subjects	Oxygen uptake l/min		Cardiac output l/min		Heart rate beats/min		Strok volume ml		(A-V) O_2-diff vol.%		Mean art. press. mm Hg		Art. O_2 content vol.%	
	bicycle	treadmill	bicycle	treadmill	bicycle	treadmill	bicycle	treadmill	bicycle	treadmill	bicycle	treadmill	bicycle	treadmill
G.H.	3.91	4.58	23.2	27.3	180	193	129	141	16.8	16.0	121	109	19.2	19 1
L.L.	4.66	4.91	24.7	24.6	187	187	132	141	18.9	18.6	110	97	21.3	20.7
J.H.	3.32	3.48	21.0	20.0	190	193	110	104	15.8	17.3	101	106	19.0	19 4
K.L.	3.44	3.53	19.2	19.3	188	190	102	102	17.9	18.3	126	104	19.9	20.3
O.S.	3.92	4.29	26.2	26.7	193	193	136	139	15.0	16.0	94	81	17 7	17.6
O.Sv.	4.54	4.61	27.6	28.3	190	193	145	146	16.5	16.3	115	102	17.8	18.0
T O.	4.59	4.58	27 7	28.8	185	191	150	151	16.6	15 9	152	136	18.5	17 9
T T	4.73	4.64	26.6	29 4	179	184	148	160	17.8	15.9	130	114	17.6	17 1
L.H.	4.23	4.74	25.6	29.0	192	196	133	148	16.5	16.3	131	104	18.9	18.3
P.S.	4.99	5.12	27.3	28.3	172	175	158	163	18.3	18.1	–	–	19.3	18.8
P-OB.	4.21	4.66	26.3	27.8	179	179	147	152	14 7	16.8	118	1*0	18.9	19 0
H.N.	4.52	4.70	26.3	28.0	189	183	136	133	16.1	16.8	138	130	19 8	19 1
C.R.	5.21	5 77	35.4	37.8	183	188	193	201	14 7	15.3	127	120	18.6	17.8
Mean	4.31	4.57	25.9	27.5	185.2	187.9	139.9	146.2	16.6	16.7	121.9	110.3	19 0	18.7
SE	0.16	0.17	1 1	1.3	1.8	1.9	6.4	7.2	0.4	0.3	4.3	4.5	0.3	0.3
SD	0.56	0.60	3.8	4.5	6.2	6.6	22.3	24.9	1 4	1 0	15.9	14.9	1 0	1 1
	$p < 0.001$		$p < 0.001$		$p < 0.01$		$p < 0.01$		$p > 0.6$		$p < 0.001$		$p < 0.01$	

Table 3. Correlations ( ) between hemoglobin concentration and maximal xygen uptake in the t tal material and in the various subgroups f students and school children.

	Maximal oxygen uptake			
	1st examination		2nd examination	
Material	l/min	ml/kg min	l/min	ml/kg x min
Total material (Students and school children)	+0.67 $p < 0.001$ (= 109)	+0.07 not signif. (n = 109)	+0.59 $p < 0.001$ (n = 208)	+0.24 $p < 0.001$ (n = 208)
Male students	−0.10 not signif. (= 20)	+0.02 not signif. (= 20)	−0.30 not signif (= 16)	−0.30 not signif. (n = 16)
Female students	−0.23 not signif. (= 21)	+0.22 not signif. (n = 21)	−0.[illegible]5 not signif. (= 15)	+0.15 not signif. (n = 15)
Schoolboys	+0.30 not signif. (37)	+0.07 not signif. (n 37)	+0.58 $p < 0.001$ (= 93)	+0.13 not signif (n = 93)
Schoolgirls	+0.55 $0.01 < p < 0.05$ (n = 31)	−0.53 not signif (n = 31)	+0.03 not signif (= 84)	+0.07 not signif (n = 84)

Table 4. Anthropometric and physiological data from the cross-sectional studies f school hildren. Mean value ± standard error of he mean and standard deviation in the different age groups are given.

Sex	Number of subjects	Age years	Height cm	Weight kg	Maximal values: Oxygen ptake l/min	Maximal values: Oxygen ptake ml/kg min	Maximal values: Heart rate beats/min	Maximal values: Pulmonary ventilation l/min	Maximal values: Blood lactate concentration mM
Female	15	11.0 ± 0.1 0.4	142.1 ± 1.9 7.0	34.9 ± 1.5 5.6	1.84 ± 0.08 0.31	53.0 ± 1 0 3.8	208.3 ± 2.1 7.8	66.3 ± 3.7 13.9	8.3 ± 0.5 2.0
Male	21	11.0 ± 0.1 0.4	140.4 ± 1.9 8.7	32.4 ± 2.8 12.4	2.02 ± 0.08 0.37	59.0 ± 1.5 6.7	202.7 ± 1.5 6.8	67.9 ± 2.8 12.6	7.5 ± 0.5 2.3
Female	38	12.0 ± 0.1 0.3	147.9 ± 1.0 7.8	40.0 ± 1 0 7.3	2.13 ± 0.05 0.34	53.7 ± 0.6 4.6	204.7 ± 1 0 7 6	72.3 ± 1 7 12.7	10.8 ± 0.5 ..3
Male	41	12.0 ± 0.1 0.3	146.7 ± 0.8 6.1	38.1 ± 0.8 6.1	2.25 ± 0.04 0.31	59 4 ± 0.7 5.1	204 7 ± 1 0 7.9	70.3 ± 1.3 11 7	9.7 ± 0.3 2.2
Female	32	13.1 ± 0.1 0.4	155.1 ± 1.2 6.5	45.3 ± 1.3 7 4	2.35 ± 0.05 0.29	52.3 ± 0.9 4.9	204 0 ± 1.3 7 1	83.6 ± 1.9 10.3	11 6 ± 0.5 2.3
Male	25	13.0 ± 0.1 0.3	151 4 ± 1.8 8.5	40.3 ± 1.2 6.0	2.32 ± 0.08 0.41	63.4 ± 1.3 6.1	204 0 ± 1.3 6.3	79.5 ± 3 1 15.3	9 6 ± 0.4 2.0
Female	1/	13.9 ± 0.1 0.3	159 1 ± 0.9 3.7	52.6 ± 1.77 6.5	2.61 ± 0.07 0.28	49.8 ± 1 7 6.6	208.4 ± 2.0 7 7	89.3 ± 2.9 11.3	11.2 ± 0.9 3.3
Male	24	13.9 ± 0.1 0.3	159 1 ± 1.8 8.8	48.8 ± 1 7 8.1	3.09 ± 0.12 0.58	63.2 ± 0.9 4.1	'03.3 ± 1.5 7.0	102.9 ± 4.4 21.3	11.5 ± 0.5 2.6
Female	12	15.0 ± 0.1 0.3	158.3 ± 1.5 5.0	51.5 ± 1.8 6.0	2.57 ± 0.11 0.35	50.2 ± 2.1 7 0	205.2 ± 2.7 9 1	90.6 ± 4.3 14 4	11.3 ± 0.9 2.8
Male	22	15.0 ± 0.1 0.3	164.6 ± 1 7 7.8	52.7 ± 1.7 7 7	3.27 ± 0.12 0.54	6..1 ± 1 1 5 1	201.8 ± 1 7 7.9	110.7 ± 4 0 18.5	13.0 ± 0.5 2.1
Female	9	16.0 ± 0.1 0.3	164.8 ± 3.5 9 8	55 0 ± 3.6 9.0	2.62 ± 0.14 0.38	47.8 ± 0.7 1.9	199.8 ± 4.0 11 4	88.6 ± 3.1 8.9	11.8 ± 1.2 3 4
Male	13	15.8 ± 0.1 0.3	169.9 ± 2.2 7.5	60.6 ± 2.0 6.8	3.83 ± 0.15 0.52	63.4 ± 1.3 4.6	202.7 ± 1.9 6.5	124 4 ± 3.2 11.2	13 6 ± 0.8 2.9

Table 5. Anthropometric and physiological data [illegible] gitudinal studies of school children in Class 4. Mean values ± standard error of the mean and standard deviation for the 6 examinations are given.

Examination	Sex	Number of subjects	Age years	Height cm	Weight kg	Maximal values: Oxygen uptake l/min	Oxygen uptake ml/kg min	Heart rate beats/min	Pulmonary ventilation l/min	Blood lactate concentration mM
1st	Female	13	12.8 ± 0.1 0.3	152.6 ± 1.7 5.8	44.1 ± 1.8 6.4	2.22 ± 0.07 0.23	50.8 ± 1.2 4.2	205.8 ± 2.9 10.2	73.6 ± 2.2 7.6	11.4 ± 0.6 1.9
	Male	16	12.7 ± 0.1 0.2	150.1 ± 1.6 6.1	40.3 ± 1.3 5.0	2.35 ± 0.08 0.29	58.4 ± 0.9 3.8	197.3 ± 2.6 10.0	64.7 ± 1.9 7.4	9.0 ± 0.6 2.4
2nd	Female	13	13.4 ± 0.1 0.3	155.8 ± 1.6 5.5	47.5 ± 2.4 8.2	2.42 ± 0.08 0.27	51.5 ± 1.3 4.8	205.1 ± 2.3 7.8	84.4 ± 3.4 12.3	12.2 ± 0.7 2.3
	Male	16	13.3 ± 0.1 0.2	155.8 ± 1.6 6.3	43.3 ± 1.4 5.2	2.59 ± 0.08 0.32	60.1 ± 1.1 4.3	200.6 ± 2.2 8.4	86.7 ± 3.0 11.8	12.3 ± 0.7 2.6
3rd	Female	13	13.8 ± 0.1 0.3	157.2 ± 1.7 6.0	49.9 ± 2.3 7.8	2.56 ± 0.06 0.21	52.0 ± 1.3 4.6	207.8 ± 1.8 6.4	88.8 ± 3.2 11.0	11.9 ± 0.7 2.5
	Male	16	13.7 ± 0.1 0.2	156.5 ± 1.7 6.7	45.7 ± 1.8 3.8	2.81 ± 0.10 0.40	63.7 ± 1.4 5.5	203.6 ± [illegible]4 9.3	95.5 ± 3.5 12.8	11.0 ± 0.5 1.9
4th	Female	13	14.2 ± 0.1 0.3	158.8 ± 1.6 5.6	51.2 ± 2.0 7.1	2.69 ± 0.07 0.25	50.9 ± 1.2 4.3	209.2 ± 2.4 8.2	95.8 ± 3.2 11.0	12.9 ± 0.4 1.5
	Male	16	14.2 ± 0.1 0.2	161.4 ± 1.9 7.3	48.2 ± 1.7 6.4	3.05 ± 0.12 0.45	63.3 ± 1.3 5.1	201.8 ± 2.0 7.7	98.3 ± 7.7 30.0	10.9 ± 0.3 1.3
5th	Female	13	14.7 ± 0.1 0.3	159.7 ± 1.8 6.1	52.6 ± 1.9 6.4	2.55 ± 0.07 0.25	48.8 ± 1.0 3.5	206.6 ± 2.2 7.5	89.6 ± 2.8 9.7	12.1 ± 0.6 2.2
	Male	16	14.7 ± 0.1 0.2	165.0 ± 1.9 7.4	52.2 ± 1.8 7.0	3.15 ± 0.11 0.43	60.1 ± 1.5 5.6	200.7 ± 2.2 8.5	104.5 ± 3.6 14.6	11.5 ± 0.6 2.3
6th	Female	13	15.2 ± 0.1 0.3	160.7 ± 1.7 6.0	53.1 ± 1.9 6.6	2.46 ± 0.07 0.25	46.6 ± 0.9 3.1	204.9 ± 1.8 6.3	89.8 ± 3.2 11.2	12.0 ± 0.7 2.4
	Male	16	15.2 ± 0.1 0.2	168.4 ± 1.8 7.1	54.6 ± 1.9 7.4	3.35 ± 0.12 0.48	61.4 ± 1.2 4.7	201.4 ± 1.9 7.5	110.0 ± 4.6 17.9	10.8 ± 0.6 2.4

Table 6. Anthropometric and physiological data from the longitudinal studies of school children in Class B. Mean values ± standard error of the mean and standard deviation for the 6 examinations are given.

Examination	Sex	Number of subjects	Age years	Height cm	Weight kg	Maximal values: Oxygen uptake l/min	Oxygen uptake ml/kg min	Heart rate beats/min	Pulmonary ventilation l/min	Blood lactate concentration mM
1st	Female	15	11.8 ± 0.1 0.3	145.7 ± 1.7 6.5	38.2 ± 1.5 5.7	2.02 ± 0.07 0.26	53.1 ± 1.1 4.1	204.8 ± 1.7 6.4	64.3 ± 2.3 6.4	11.2 ± 0.4 1.4
	Male	14	12.0 ± 0.1 0.3	145.4 ± 1.6 5.8	37.4 ± 2.0 7.1	2.18 ± 0.06 0.21	58.1 ± 2.0 7.2	208.4 ± 1.6 5.9	64.0 ± 2.3 8.5	9.7 ± 0.4 1.5
2nd	Female	16	12.3 ± 0.1 0.3	149.5 ± 1.7 6.4	41.8 ± 1.7 6.4	2.24 ± 0.07 0.25	54.2 ± 1.4 5.3	[illegible]05.6 ± 2.0 7.5	80.5 ± 2.4 9.0	12.4 ± 0.6 2.1
	Male	14	12.5 ± 0.1 0.3	148.6 ± 1.8 6.5	39.5 ± 2.0 7.1	2.59 ± 0.07 0.[illegible]7	61.4 ± 1.8 6.5	206.9 ± 1.6 5.6	74.1 ± 3.1 11.5	11.6 ± 0.5 1.9
3rd	Female	16	12.8 ± 0.1 0.3	152.1 ± 1.7 6.2	44.6 ± 1.7 6.4	2.42 ± 0.07 0.27	54.7 ± 1.5 5.4	[illegible]08.5 ± 1.8 6.7	86.8 ± [illegible] 10.9	11.8 ± 0.5 2.0
	Male	14	13.0 ± 0.1 0.3	151.2 ± 2.1 7.7	41.8 ± 2.1 7.7	2.87 ± 0.10 0.36	64.6 ± 1.7 6.0	210.6 ± 1.6 5.6	83.0 ± 3.7 13.2	12.8 ± 0.6 2.3
4th	Female	15	13.3 ± 0.1 0.3	155.3 ± 1.6 6.0	46.9 ± 1.6 5.9	2.41 ± 0.06 0.21	51.8 ± 1.3 5.0	208.8 ± 2.4 8.9	89.6 ± [illegible] 9.5	12.5 ± 0.5 1.8
	Male	14	13.5 ± 0.1 0.3	155.7 ± 2.4 8.6	45.5 ± 2.2 7.8	2.69 ± 0.12 0.42	62.3 ± 1.2 4.4	206.6 ± [illegible] 7.2	[illegible] ± 5.6 [illegible]0.3	11.9 ± 0.5 1.8
5th	Female	16	13.8 ± 0.1 0.3	157.9 ± 1.8 5.6	50.1 ± 1.6 5.8	2.48 ± 0.04 0.17	48.8 ± 1.2 4.3	[illegible]04.7 ± 2.2 8.3	85.6 ± [illegible] 8.9	11.0 ± 0.7 2.7
	Male	14	14.0 ± 0.1 0.3	160.1 ± 2.6 9.5	48.1 ± [illegible] 8.9	2.99 ± 0.14 0.51	62.6 ± 1.6 5.7	[illegible]06.3 ± 1.5 5.4	97.4 ± 3.9 21.4	11.4 ± 0.3 1.1
6th	Female	16	14.3 ± 0.1 0.3	160.5 ± 1.5 6.3	50.8 ± 1.4 5.2	2.50 ± 0.01 0.17	49.6 ± 1.3 4.7	[illegible]04.9 ± 1.7 6.3	98.4 ± 2.6 9.8	10.7 ± 0.6 2.3
	Male	14	14.5 ± 0.1 0.3	163.4 ± 2.3 9.1	49.8 ± 2.6 9.2	3.09 ± 0.16 0.56	62.3 ± 1.5 5.8	203.1 ± 1.4 5.2	102.2 ± 6.1 22.1	10.8 ± 0.6 2.2

Table 7 Anthropometric and physiological data describing the ntrained ad lt subjects. Mean values ± standard error of the mean and standard deviation of the different age group are given.

Sex	Number of subjects	Age group	Height cm	Weight kg	Maximal values Oxygen uptake l/min	ml/kg min	Heart rat beats/min	Pulmonary ventilation l/min	Blood lactate concentration mM
Female	14	20–29	169 4 ± 1.3 4.6	60.8 ± 1.8 6.6	2.41 ± 0.07 0.27	37.9 ± 0.8 2.8	205.6 ± 2.6 9.5	81 6 ± 2.[illegible] 8.0	11.5 ± 0.5 1 7
Male	20	20–29	181.8 ± 1.3 5.8	73.8 ± [illegible].0 8.7	3.96 ± 0.11 0.47	54 1 ± 1.5 6.7	201.3 ± 2.0 8.8	145.8 ± 3.9 16.9	12.8 ± 0.6 2.4
Female	8	30–39	165.4 ± 2.7 7.2	63.3 ± 3.0 8.0	2.09 ± 0.09 0.[illegible]5	33.2 ± 1.5 3.9	185.3 ± 2.3 6.0	60.0 ± 3.8 10 0	–
Male	24	30–39	178.2 ± 1.2 5.6	76.1 ± 1 7 8.1	3.16 ± 0.08 0.39	41.8 ± 1 1 5 0	189 9 ± 1.8 8.8	90.0 ± 3 7 17 6	–
Female	14	40–49	165.2 ± 1 7 6.2	59.8 ± 2.1 7 4	1.95 ± 0.07 0.26	32.9 ± 1.3 4.5	178.4 ± [illegible].5 9 0	56.7 ± 2.7 9.7	–
Male	16	40–49	179.0 ± 1 4 5.5	75.1 ± 2.5 9.5	2.93 ± 0.09 0.34	39.3 ± 1 0 4 0	188.8 ± 3.4 13.2	83.5 ± 4 0 15 4	–
Female	8	50–59	163.9 ± 1.9 5.1	64.3 ± 4 4 11 7	1.81 ± 0.10 0.27	28.5 ± 1.8 4.7	171.8 ± 4 7 12.5	60.7 ± 5 1 13.5	–
Male	17	50–59	177 1 ± 2.0 7.9	72.1 ± 3.1 12.3	2.58 ± 0.10 0.41	36.1 ± 1 1 4.3	177 1 ± 3.9 15 7	76.2 ± 3.6 14.4	–

Table 8. Pertinent data describing the female physical education students and the different groups of female athlet s.

Sports event	Number of subjects	Age years	Height cm	Weight kg	Maximal values: Oxygen uptake l/min	Oxygen uptake ml/kg x min	Heart rat beats/min	Pulmonary ventilation l/min	Blood lactat concentration mM
Orienteering	6	23.3 ± 2.4 6.4	168.8 ± 1 7 3.8	60.0 ± 1.9 4.3	3.65 ± 0.3 0.66	60.7 ± 3.3 7 4	184 2 ± 5.0 11.2	115 1 ± 10.1 22.5	12.5 ± [illegible]5 3.2
Running 400–1500 m	5	21 4 ± 2.0 4.0	170.2 ± 0.7 1.5	58.6 ± 1.8 3.6	3.36 ± 0.14 0.29	57.3 ± 1.5 3.1	197 6 ± 4.5 9 0	102.5 ± 4.5 9 0	10.8 ± 0.7 1 1
Skiing cross-country	9	25.2 ± 1.8 5.5	165.7 ± 2.1 6.4	60.6 ± 1 4 4.2	3.44 ± 0.08 0.23	56.9 ± 1 0 2.9	191 1 ± 2.8 7.8	106.4 ± 3.8 11 4	11 1 ± 1 2 3 4
Speed skating	8	20.4 ± 1.6 4.1	168.1 ± 1.8 4.8	65.4 ± 2.0 5 2	3.38 ± 0.07 0.18	52.0 ± 1.6 4 2	201.6 ± 3.6 9.5	101.[illegible] ± 3.2 8.5	10.3 ± 0.9 2.3
Gymnastics	11	15.5 ± 0.5 1 6	159 7 ± 1.9 6.1	48.8 ± 2.1 6.5	2.42 ± 0.09 0.28	49 8 ± 1 1 3.5	195.6 ± [illegible].0 6.4	87.3 ± 4.3 14.1	15.5 ± 0.5 1 6
Phys.educat. students	21	22.2 ± 0.4 1 7	169.6 ± 1.5 6.9	59.3 ± 1 7 7 4	2.71 ± 0.08 0.35	46.0 ± 1 2 5.2	198.9 ± 1.5 6.9	90.4 ± 2.4 10.7	13.6 ± 0.5 2.3

Table 9 Pertinent data describing the male physical education students and the different groups of male athletes.

Sports event	Number of subjects	Age years	Height cm	Weight kg	Maximal values: Oxygen uptake l/min	Oxygen uptake ml/kg x min	Heart rate beats/min	Pulmonary ventilation l/min	Blood lactate concentration mM
Skiing cross-country	6	25.4 ± 1.5 2.5	180.4 ± 2.4 4.8	73.2 ± 2.3 4.7	5.41 ± 0.07 0.14	73.9 ± 1.7 3.4	186.2 ± 2.7 5.4	157.9 ± 9.5 18.9	11.0 ± 1.6 2.8
Running long distance	8	26.4 ± 1.2 3.2	176.1 ± 1.9 4.9	64.5 ± 2.0 5.2	4.67 ± 0.15 0.40	72.2 ± 0.8 2.0	191.8 ± 2.9 7.7	145.7 ± 3.3 8.8	14.9 ± 0.7 1.5
Orienteering	16	24.6 ± 0.9 3.3	179.7 ± 1.0 3.8	70.3 ± 2.0 7.9	4.96 ± 0.08 0.31	71.1 ± 1.5 5.8	191.3 ± 2.1 8.1	160.7 ± 3.2 12.3	12.8 ± 0.7 2.2
Bicycling	7	24.1 ± 1.3 3.1	180.4 ± 2.3 8.5	79.2 ± 2.2 5.4	5.55 ± 0.14 0.34	70.3 ± 2.4 5.9	184.9 ± 2.5 6.1	164.5 ± 10.1 4.8	10.6 ± 0.9 [illegible]1
Canoeing	2	22.5	190.5	80.7	5.49	67.7	190.5	165.0	16.4
Rowing	12	24.8 ± 0.6 1.8	189.9 ± 1.0 3.5	86.9 ± 1.4 4.7	5.80 ± 0.07 0.22	66.9 ± 0.9 2.8	189.3 ± 2.1 6.9	179.7 ± 3.7 12.3	10.1 ± 1.0 2.5
Speed skating	7	24.7 ± 1.8 4.4	183.1 ± 2.4 5.9	82.4 ± 2.8 6.7	5.33 ± 0.29 0.72	64.6 ± 2.4 5.8	194.0 ± 5.5 13.5	157.9 ± 9.9 24.3	14.3 ± 1.9 4.6
Handball	13	24.4 ± 0.8 2.8	183.7 ± 1.2 4.3	81.3 ± 2.3 7.8	4.88 ± 0.17 0.6	60.0 ± 1.4 4.8	189.3 ± 3.1 10.8	145.5 ± 4.7 16.4	14.5 ± 0.8 2.5
Bandy	15	24.3 ± 1.2 4.4	178.4 ± 1.7 6.3	75.4 ± 1.7 6.3	4.22 ± 0.11 0.40	56.1 ± 1.3 4.9	189.1 ± 2.1 8.0	127.3 ± 3.8 14.4	11.2 ± 0.7 2.7
Phys.educat. students	20	24.4 ± 0.5 2.3	180.3 ± 1.7 7.3	76.0 ± 2.1 8.9	4.72 ± 0.14 0.62	62.4 ± 1.7 7.5	192.1 ± 1.3 5.5	146.1 ± 4.5 19.6	14.2 ± 0.6 2.8

ACTA PHYSIOLOGICA SCANDINAVICA
Supplement 400

EXPERIMENTAL AND CLINICAL STUDIES ON THE THROMBIN LIKE ENZYME FROM THE VENOM OF BOTHROPS ATROX. ON THE PRIMARY STRUCTURE OF FRAGMENT E

1

by

Nils Egberg

Stockholm 1975

ACTA PHYSIOLOGICA SCANDINAVICA
Supplement 400

Department of Blood Coagulation Research Karolinska Institutet, Blood Coagulation Laboratory and Thoracic Surgery Research Laboratory Karolinska Sjukhuset, Stockholm Sweden

EXPERIMENTAL AND CLINICAL STUDIES ON THE THROMBIN LIKE ENZYME FROM THE VENOM OF BOTHROPS ATROX. ON THE PRIMARY STRUCTURE OF FRAGMENT E

by

Nils Egberg

Stockholm 1975

Tryckt hos GEO-TRYCK AB Stockholm 1973

The present dissertation comprises a summary of the following separately published papers

I Egberg N and S Nordström, Effects of Reptilase induced intra vascular coagulation in dogs. *Acta physiol. scand.* 1970 *79* 493–505

II Egberg N and A Ljungqvist, On fibrin distribution in organs of dogs during defibrination with the thrombin like enzyme from Bothrops atrox *Thrombos Res* 1973 *3* 191–207

III Egberg N and H Johnsson, Platelet aggregation induced by ADP and thrombin in Reptilase defibrinated dogs. *Thrombos Res* 1972 *1* 95–112

IV Egberg N Coagulation studies in patients treated with Defibrase *Acta med scand* In Press.

V Egberg N On the metabolism of the thrombin-like enzyme from the venom of Bothrops atrox. *Thrombos. Res.* In press.

VI Kowalska Loth, B B Gårdlund N Egberg and B Blombäck, Plasmic degradation products of human fibrinogen II Chemical and immunological relation between fragment E and N-DSK. *Thrombus Res.* 1973 2 423–450

CONTENTS

Preparations of clot promoting enzymes from the venom of Bothrops atrox mentioned in this report. 7
Introduction 9
In vivo effects of the thrombin-like enzymes from Bothrops atrox studied in dogs (I and II) 11
Previous investigations 11
Present investigations 12
Experimental conditions 13
The process of fibrinogen depletion (I) 15
The process of fibrin removal (II) 17
Summary and conclusion 18
Influence of the thrombin like enzyme from Bothrops atrox on the haemostatic mechanism (III and IV) 19
Previous investigations 19
Present investigations 20
Experimental and therapeutic conditions 20
The influence of Defibrase on platelet function (III) 21
Coagulation studies in patients treated with Defibrase (IV) 23
Summary and conclusion 25
On the metabolism of the thrombin-like enzyme from Bothrops atrox (V) 27
Previous investigations 27
Present investigations 27
In vitro studies 28
In vivo studies 30
Summary and conclusion 32
On the primary structure of Fragment E (VI) 34
Previous investigations 34
Present investigations 34
Experimental conditions 35
Chemical and immunological relation between Fragment E and N DSK 35
Summary and conclusion. 37
General summary 38
Acknowledgements 40
References 42

PREPARATIONS OF CLOT PROMOTING ENZYMES FROM THE VENOM OF BOTHROPS ATROX MENTIONED IN THIS REPORT

All preparations are manufactured by Pentapharm AG Basle Switzerland

1 Reptilase (Haemocoagulase) Available as 1 ml ampoules. The enzymatic activity of this preparation is 1 Klobusitzky unit[a)] per ml and the clot-promoting activity is derived from a mixture of thrombin like and thromboplastin like enzymes.

2 Reptilase R. Lyophilized preparation containing only the thrombin-like enzyme(s) together with stabilizers to a total amount of 34 mg per ampoule The clotting activity is equivalent to about 0 1 NIH units of thrombin per mg The preparation is meant for laboratory use only

3 Defibrase (initially named Reptilase DEF) Sterile preparation containing only the thrombin-like enzyme(s) together with preservative. Defibrase is available as 2 ml ampoules and is intended for clinical use Its enzymatic activity is equivalent to about 3.5 NIH units of thrombin per ml. Recent studies have revealed that the clot-promoting enzymes from Bothrops venom for many years have been prepared from two different subspecies Bothrops marajoensis and Bothrops moojeni. The thrombin-like enzymes from these two subspecies are not identical. For studies mentioned in this report batches 405 416 460 477 500 and 550 were used. All batches except 550 contained a mixture of Bothrops marajoensis and Bothrops moojeni enzyme. Batch 550 contained Bothrops moojeni enzyme

4 Highly purified thrombin-like enzyme. Lyophilized preparations prepared from the venom of Bothrops marajoensis denoted K 126 and DG 144 VIII–XIII The thrombin-like activity of these preparations were 95 and 120 NIH units of thrombin per mg respectively

a) One Klobusitzky unit is defined as the quantity of Reptilase which diluted to 1 ml in 0 15 M buffer at neutral pH coagulates 5 ml of oxalated horse blood in 8–10 minutes t 20–22°

INTRODUCTION

It has been demonstrated that several snake venom enzymes possess blood coagulant properties (Mellanby 1909 Eagle 1937 Nahas Denson and Macfarlane 1964 Copley Banerjee and Devi 1973) The clot promoting enzymes of these snake venoms have been found to exert their action on blood coagulation by two different mechanisms (Eagle 1937 Nahas Denson and Macfarlane 1964) Thus one group of enzymes accelerates the conversion of prothrombin to thrombin (thromboplastin like enzymes) while another group causes a direct transformation of fibrinogen to fibrin (thrombin-like enzymes)

Eagle (1937) demonstrated that the venoms of the two South American snakes Fer de lance and Jararaca or Bothrops atrox and Bothrops jararaca respectively contained both thrombin and thromboplastin-like enzymes. A method for the isolation of the clot-promoting principle from the venom of Bothrops jararaca was described by v Klobusitzky (1935) and by v Klobusitzky and König (1936) A preparation isolated according to this method was later commercially marketed as Reptilase and found to contain both the thromboplastin and the thrombin like enzymes from the Jararaca venom (Deutsch 1955 Zurn 1959) By further purification of the Reptilase preparation the thrombin-like enzyme was isolated (Stocker 1970) It was shown that the purified thrombin-like enzyme (initially also named Reptilase) was not inhibited by heparin in the presence of heparin co-factor (Hohnen 1957 Blombäck Blombäck and Nilsson 1957) By means of NH_2 terminal analysis Blombäck (1958) and Blombäck and Laurent (1958) demonstrated *that the thrombin-like enzyme clotted fibrinogen* by splitting off only fibrinopeptide A in contrast to thrombin which removes both fibrinopeptides A and B It was also shown that the fibrin formed by this thrombin like enzyme initially polymerizes only end-to-end whereas thrombin fibrin polymerizes both end to-end and side-to-side (Laurent and Blombäck 1958)

The purified form of Reptilase *per se* did not produce fibrinolytic activity when tested on fibrin plates (Blombäck, Blombäck and Nilsson 1957)

IN VIVO EFFECTS OF THE THROMBIN-LIKE ENZYMES FROM BOTHROPS ATROX STUDIED IN DOGS (I AND II)

Previous investigations

In 1936 v Klobusitzky and König suggested that the clot-promoting fraction of the Bothrops jararaca venom could possibly be used for therapeutical purpose in the treatment of haemophilic patients. Several studies on the haemostatic effect of Reptilase (cf. page 7) has since been performed It has been claimed that in normal subjects injections of Reptilase promptly causes a decrease of both the clotting and bleeding times (Heiss 1954 Bruck 1957 Stacher and Böhnel 1959 Steinhoff 1960) These effects of Reptilase have been used therapeutically in the treatment of patients with haemorrhagic diatheses (Fleischhacker 1954 Ohlenschläger and Schwalbe 1966) and prophylactically as a haemostatic agent in connection with surgical interventions such as tonsillectomy and prostatectomy (Durante et al. 1962 Sugano and Maruyama 1964) in otherwise normal patients.

The doses of Reptilase given have mostly been 0 25–2 Klobusitzky units once or twice during a 24-hour period (Bruck 1957 Steinhoff 1960 Durante et al 1962) Depending on the mode of administration, the maximum effect on coagulation and bleeding times is obtained within 3–20 minutes (intravenous administration giving the most rapid effect) and it has been claimed that the effect lasts for at least 24 hours (Steinhoff 1960) Apart from the effect of Reptilase on bleeding and coagulation times no marked influence was found in normal subjects on the platelet count or the concentrations of factor V and VII (Stacher and Böhnel 1959) These findings indicate that only a low graded intravascular coagulation was induced by the given doses of Reptilase. However Tóth et al. (1967) and Tóth Lévai and Szilágyi (1970) showed that administration of 1–2 Klobusitzky units of Reptilase in connection with the challenge dose of endotoxin markedly increased the intensity of local as well as of generalized Schwartzman s phenomenon

Reid Chan and Thean (1963) reported on observations in humans bitten by the Malayan pit viper The victim s blood was found to

remain incoagulable for 2–14 days though no bleeding symptoms were observed The venom from Agkistodon rhodostoma was later shown to contain a thrombin like activity (Nahas Denson and Macfarlane 1964 Chan 1964 Chan Rizza and Henderson 1965) In rabbits the Agistrodon rhodostoma venom rapidly produced a depletion of the circulating fibrinogen apparently without affecting other parts of the coagulation mechanism and without thrombus formation (Regoeczi Gergely and McFarlane 1966) The thrombin-like enzyme was later purified (Esnouf and Tunnah 1967) and commercially named Arvin. Marshal and Esnouf (1968) reported that Arvin injections produced a rapid depletion of fibrinogen in dogs and that dogs defibrinated (in text used equivalent to defibrinogenation) with Arvin were prevented from developing thrombosis when experimental induction was attempted. No toxis side effects were observed after injections of Arvin to dogs. They furthermore showed that injections of large doses of Arvin (4 times the ordinary dose) did produce thrombus formation in the right heart and pulmonary arteries It has also been shown that impairment of the fibrinolytic system by injection of fibrinolytic inhibitors, prior to the administration of normally harmless doses of Arvin, markedly increases the risk of severe organ damage (Silberman, Potter and Kwaan 1971 Kwaan and Barlow 1971) The fibrinolytic system thus appears to be of major importance for the removal of fibrin deposits after Arvin injections. Kwaan and Barlow (1971) *in vitro* showed that clots formed by Arvin or Reptilase are more readily digested by plasmin than clots formed by thrombin. Esnouf and Marshall (1968) showed that the fibrin clearing effect of the reticuloendothelial system (RES) did not seem to be as essential in the case of Arvin induced defibrination as in the case of thrombin induced intravascular coagulation (Lee 1962)

Present investigations

The purpose of the first *in vivo* studies on Defibrase was to determine the appropriate dose for uncomplicated defibrination in dogs and to follow the course of defibrination (I) The following study (II) was devoted to a histological examination of the fibrin distribution in organs from dogs defibrinated under conditions similar to those in the previous investigation.

The preparations of purified Bothrops venom were standardized

according to their thrombin-like activity on fibrinogen (Blombäck and Blombäck 1963)

At the time of the first study 1969 (I) the thrombin like enzyme of Bothrops atrox was only available as Reptilase R (cf page 7) while in the second study (II) the more convenient preparation Defibrase (cf page 7) was used. These two preparations are identical with regard to thrombin like enzymes (Stocker 1972) In both studies ^{125}I-labelled fibrinogen was used to follow the process of fibrinogen depletion (I) and to perform autoradiographic examinations (II)

Dog fibrinogen used in the studies was prepared by two different techniques (Blombäck and Blombäck 1956 Blombäck Blombäck and Holmberg 1966 Blombäck and Blombäck 1966) Both methods gave a product with a coagulability of about 98 %. The dog fibrinogen was labelled with ^{125}I isotope according to the method described by McFarlane (1963) The coagulability of the fibrinogen after labelling was about 90 %. The specific activity was about 2μCi/mg in the first study (I) and about 13 μCi/mg in the second (II) and the labelling approximately 1 atom of iodine per mole of fibrinogen. The biological half lives of some of these ^{125}I fibrinogen preparations were graphically calculated and found to be about 2.2 days, which is in agreement with earlier observations (Nordström and Zetterqvist 1968 1969)

Dog fibrinogen prepared as above was also used for immunization of rabbits to produce dog fibrinogen antiserum. The immunoglobulins of this antiserum were precipitated at 1/3 saturation of ammonium sulphate after which the immunoglobulinfraction was subjected to labelling with fluoresceinisothiocyanate according to Bergqvist and Schilling (1970)

Experimental conditions

The first study (I) In order to reveal dose response relationship and to perform preliminary determinations of the influence on blood pressure pulse rate and body temperature six dogs were given various doses of Reptilase–R,7 7–0.9 mg/kg b w administered intravenously during one hour Twelve dogs were thereafter given a dose of 1 7 mg/kg b w (cf page 7) intravenously during one hour After previous injection of ^{125}I fibrinogen (average 3.8 μCi/kg b w) the animals divided between four experimental groups

Group No	Administered drugs
I	Reptilase R
II	Reptilase R + Heparin
III	Reptilare-R + ϵ-aminocaproic acid
IV	Reptilase R + Trasylol

One dog out of Group III and one out of Group IV were after the initial infusions given further treatment with Reptilase R by one daily intravenous or intramuscular injection for 10 and 16 days respectively

Prior to and during the initial infusion of Reptilase R all dogs were anaesthetized by intravenous injections of thiopenthal sodium Dogs given long-term treatment with Reptilase R were given the second and subsequent injections rapidly and without anaesthetia.

Blood samples were collected at intervals for determinations of plasma fibrinogen and serum-radioactivity fibrinogen concentration and platelet count. Factor V and prothronbin concentrations were determined in one and two dogs respectively Immunodiffusion and immunoelectrophoretic studies were performed in some dogs on serum samples for detection of fibrin(-ogen) degradation products

The second study (II) In order to provoke if possible a thrombus formation 16 anaesthetized dogs were given a rapid injection of a dose corresponding to the double standard dose of the previous study (0 1 ml of Defibrase/kg b w equivalent to 0.35 NIH units of thrombin to 3.5 mg of Reptilase–R per kg b w)

Nine dogs analyzed by autoradiographic technique were pretreated with ^{125}I fibrinogen (average dose 38 4 μCi/kg b w) 1–2 hours before the experiment Eight dogs (including three of the dogs analyzed by autoradiography) were examined by immunofluorescent technique One of the dogs examined by immunofluorescence had been defibrinat ed by one daily injection of Defibrase (0 1 ml/kg b w) for 7 days before the experiment.

The dogs were sacrificed at various intervals 1/2–6 hours after the Defibrase injections by injection of a large dose of thiopenthal sodium. Immediately after death specimens of lung liver spleen, kidney and sceletal muscle were removed for histological examination.

Cryostate sections were prepared for both autoradiographic and immunofluorescent examinations Due to the relatively low doses of radioactivity used thick sections (20–60 mm) were employed and autoradiographs were prepared according to the contact method described by Gross et al (1951) Sections were prepared for observation of direct immunofluorescence in UV light.

Two control dogs were given physiological saline and thrombin (125 NIH units per kg b w during 30 minutes) respectively and sacrificed 30 minutes after the start of the injections. The dog given physiological saline was examined by both autoradiography and by immunofluorescence. The dog given thrombin was only examined by immunofluorescence in order to test the reactivity of the fluorescein-conjugated antibody in histological sections

In addition to these histological investigations the clearance of endogenous creatinine was determined in four experimental animals (two of these dogs were also used for histological examinations) and in the control dog given physiological saline. Determinations were performed at 30-minute intervals before and after the Defibrase injections.

In all of the dogs given ^{125}I fibrinogen except one the urinary excretion of labelled material was continuously determined.

The process of fibrinogen depletion (I)

The largest dose of Reptilase R (7 7 mg/kg b w) caused a complete fibrinogen depletion within two hours while the smallest dose (0 9 mg/kg b w) caused a 50 % decrease of the fibrinogen concentration within six hours. At the standard dose of 1 7 mg/kg b w a decrease by about 80 % of the initial fibrinogen consentration was obtained within six hours in animals from group I On the day after the experiment the fibrinogen level was essentially the same as six hours after the Reptilase-R infusion and the pre infusion value was only reached in one dog within three days

Fibrinogen radioactivity showed a decrease parallell to the fibrinogen concentration. Plasma radioactivity initially fell parallell to the fibrinogen radioactivity but after one hour levelled off simultaneously with an increase of the serum radioactivity Immunoelectrophoresis of serum sample taken one hour after the Reptilase R infusion revealed presence of fibrin(-ogen) degradation products (FDP) Obviously FDP re-entering the circulation caused the slow decrease of plasma radioactivity and the increase of serum radioactivity

The platelet count decreased more than 40 % in the dog given the largest dose of Reptilase-R, while at the standard dose the platelet count fell about 25 % (Group I) Factor V activity decreased by about 75 % within seven hours in the dog examined. The prothrombin on the other hand, was unaffected in one dog and decreased only

about 30 % in the other The decrease of factor V may be due to methodological problems but may also depend on an activation caused by contaminant thromboplastin like enzyme in Reptilase R. The decrease of factor V may also be due to intravascular coagulation, but other indications of such a phenomenon were not observed (clinical signs decrease of platelet count)

In dogs given simultaneous infusions of Reptilase R and heparin (Group II) the course of defibrination and the appearence of FDP was similar to those in dogs from Group I indicating that even *in vivo* heparin does not inhibit the effect of Reptilase R. The platelet count however seemed to be essentially unaffected in this group of animals. The moderate decrease of the platelet count in animals of Group I might be due to aggregation of platelets by traces of thromboplastin like enzyme present in Reptilase R causing an endogeneous generation of thrombin that induces platelet aggregation In the presence of heparin such an aggregation would be inhibited.

Simultaneous infusions of Reptilase R and fibrinolytic inhibitors, ϵ-aminocaproic acid and Trasylol respectively (Group II and IV) were found to delay but not to inhibit completely the secondary fibrinolytic process. Fibrinolytic degradation products were thus demonstrated immunologically 2—4 hours after the Reptilase R injec tion The incomplete inhibition of the degradation of fibrin(-ogen) by the drugs administered might be due to an inadequate inhibition of the rinolytic system or to an enzymatic degradation of fibrin(-ogen) not affected by ϵ-aminocaproic acid or Trasylol

ϵ-aminocaproic acid seemed to potentiate the defibrinating effect of Reptilase-R since two of the three dogs were completely defibrinated within six hours Platelet count was also less affected in the ϵ-aminocaproic acid and Trasylol group (Groups III and IV) than in the reference group given only Reptilase R (Group I) The latter finding might be due to a thromboplastin inhibiting effect of these compounds previously observed by Nordström and Zetterqvist (1969) No signs of thrombo-embolism were demonstrable in any of the dogs at autopsy Simultaneous administration of Reptilase R and fibrinolytic inhibitors thus did not seem to severely inhibit the removal of fibrin deposited in the vascular bed during defibrination This is in conformity with observations of Reid (1965) on patients bitten by the Malayan pit viper On the other hand several authors have observed thrombo-embolic complications in rabbits given simultaneous injections of EACA and Agkistrodon rhodostoma venom or Arvin (Regoeczi

Gergely and McFarlane 1966 Silberman Potter and Kwaan 1971 Kwaan and Barlow 1971) This descrepancy might possibly be due to species differences or to variations in the doses given.

Dogs given long term Reptilase R treatment could be kept at fibrinogen concentrations below 1 0 mg/ml by one daily injection of Reptilase R Intramuscular administration appeared to be less effective than intravenous

The process of fibrin removal (II)

In a few dogs sacrificed 30 minutes and one hour after the Defibrase injections minor fibrin deposits were demonstrated by immunofluorescence in the lung capillaries On the other hand the glomerulies of the kidneys were not affected in any of the dogs. In agreement with this finding it was further demonstrated that the glomerular filtration rate as measured by clearence of endogenous creatinin did not seem to be significantly affected during the early phase (initial five hours) of Defibrase defibrination

By the immunoflourescent technique it was also demonstrated that in a few of the dogs sacrificed 30 minutes to five hours after Defibrase injection small amounts of flourescent material was to be found in the liver sinusoides. Even in the dog sacrificed after eight days of defibrination fibrinogen related material (FR-material) was reveled in the liver sinusoides.

Autoradiographs did not reveal any intravascular fibrin deposits possibly due to a too high background radioactivity In one dog killed 30 minutes and two dogs killed one hour after the Defibrase injection radioactive material appeared to be accumulated in the red pulp or the spleen at the periphery of the Malpigian corpuscles. These dogs were not examined by immunoflourescent technique

The fact that only small amounts of FR-material were found either in the spleen or in the liver indicates that during defibrination induced by Defibrase the removal of FR material by RES is of only minor importance. In accordance with these findings, Marshal and Esnouf (1968) demonstrated that the removal of FR material by the RES was of minor importance in dogs after Arvin administration since the incidence of thrombo-embolism did not increase after blockade of the RES

The secondary fibrinolysis obtained during Defibrase induced defibrination would thus be of major importance for the removal of fibrin. About 80 % of the administered radioactivity was excreted in the urine whithin eight days By means of immunoelectrophoresis urine samples were shown to contain FR material (possibly E fragments) giving precipitin reactions with anti-dog fibrinogen serum which also would indicate that the fibrinolytic process is mainly responsible for the removal of fibrin In accordance with these findings Regoeczi, Gergely and McFarlane (1966) Silberman, Potter and Kwaan (1971) and Kwaan and Barlow (1970) found that the incidence of thrombo-embolic complications increased drastically when Arvin was administered to rabbits treated with fibrinolytic inhibitors.

Summary and conclusion

In the first study the elimination of ^{125}I labelled fibrinogen was investigated in dogs after injections of Reptilase R (cf page 7)

By intravenous administration of Reptilase R, a total depletion of the circulating fibrinogen could be accomplished in dogs within two to six hours without provoking signs of vascular obstruction bleedings or toxic reactions. A minor decrease of the platelet count was observed with heparin did not affect the defibrination process secondary to the defibrination a fibrinolytic process was induced. This fibrinolysis seemed not to be completely inhibited by administration of fibrinolytic inhibitors. No thrombo-embolic complications were observed in dogs given fibrinolytic inhibitors.

In the second study Defibrase (cf. page 7) was injected intravenously *to dogs that were sacrificed 1/2–6 hours later Sections from lung* liver kidney and spleen were examined by means of autoradiography and immunoflourescent techniques. The observations in the previous study were supported by the histological examinations. Only minor fibrin deposits could be demonstrated in the lung capillaries in a few dogs The glomerular filtration rate was not markedly affected by Defibrase injections indicating that the defibrination procedure did not produce severe circulatory disturbances. Only small amounts of fibrinogen related material was demonstrated in the liver or the spleen suggesting a minor importance of the reticuloendothelial system for the removal of fibrin after Defibrase injections.

INFLUENCE OF THE THROMBIN-LIKE ENZYME FROM BOTHROPS ATROX ON THE HAEMOSTATIC MECHANISM (III AND IV)

Previous investigations

In vitro studies on platelet aggregation has discovered that presence of small amounts of fibrinogen is necessary for the normal platelet aggregation induced by ADP (Cross 1964 Solum and Stormorken 1965) An inhibitory effect on ADP induced platelet aggregation by FDP has been reported (Kowalski Kopec and Wegrzynowicz 1963 Larrieu, Inceman and Marder 1967) However other workers have not found such an inhibitory effect by FDP (Hirsh, Fletcher and Sherry 1965 Cronberg 1968) and it has even been claimed that FDP induces platelet aggregation (Barnhart et al. 1967)

The preparation of Bothrops venom enzymes namned Reptilase (cf page 7) was found to induce platelet aggregation and release of ADP (Morse 1967) Reptilase did however not produce clot retraction, which was suggested to be due to absence of release of fibrinopeptide B Reptilase (1–2 Klobusitzky units) administered to patients was found to produce shortened bleeding times (Stacher and Böhnel 1959) They furthermore stated that Reptilase caused a shortening of whole blood coagulation time but only slightly affected the concentrations of different coagulation factors

Arvin did not affect the concentrations of coagulation factors *in vitro* (Bell, Bolton and Pitney 1968) and in view of earlier findings in animal experiments the therapeutic effect of defibrination induced by Arvin was investigated in patients with thrombo-embolic disorders. From these studies it was reported that Arvin treatment did not affect the platelet count (Bell, Pitney and Goodwin 1968 Sharp et al. 1968) Prentice et al. (1969) however found that the ADP induced platelet aggregation was decreased during the first 24 hours of Arvin treatment but then returned to about pretreatment level. The decrease of the platelet aggregation was concommitant with maximum concentrations of FDP and was thus considered to be due to the inhibitory effect by FDP

Arvin infusions were found to cause a rapid decrease of the fibrinogen concentrations but not to produce any decrease of other coagulation factors (Bell Pitney and Goodwin 1968 Sharp et al. 1968) During Arvin treatment a decrease of the plasminogen concentrations was observed (Bell Pitney and Goodwin 1968 Sharp et al. 1968)

The thrombin like enzyme from Bothrops atrox unlike Arvin has in vitro been shown to activate the fibrin stabilizing factor factor XIII (Barlow Holleman and Lorand 1970 Lorand 1972) Recent investigations on the thrombin like enzymes from the two different subspecies Bothrops marajoensis and Bothrops moojeni have revealed that only the enzyme from the latter subspecies activates factor XIII (McDonagh and McDonagh 1973) The batches of Defibrase (403 416) used for treatment of the three patients in whom factor XIII was determined (see below) contained a mixture of enzymes from these two subspecies (Stocker 1973)

Present investigations

The purpose of the present studies was to disclose the effect of Defibrase induced defibrination on different haemostatic mechanisms. the first study (III) the platelet function during Defibrase induced ~brination and in the defibrinated state was investigated in dogs. The ~ects on different coagulation factors on plasminogen, α_2 macroglobulin and antithrombin III levels were recorded in the second study IV) by determinations in patients treated with Defibrase for various hrombotic disorders.

Experimental and therapeutic conditions

Seventeen anaesthetized and non anaesthetized dogs (four and thirteen dogs respectively) were injected with 0 10 ml of Defibrase per kg b w (III) Platelet rich plasma (PRP) obtained from consecutive blood samples was subjected to analysis of platelet aggregation induced by ADP and thrombin The effect of reestablishing normal fibrinogen concentration of these samples in vitro was also studied. Bleeding times were determined in three dogs

In order to reveal the effect of FDP on ADP induced platelet aggregation fibrinogen containing plasminogen was digested *in vitro* by addition of urokinase after which the fibrinogen digests were infused to one non-defibrinated and anaesthetized and one defibrinated and non-anaesthetized dog Platelet aggregation was followed after infusion of the fibrinogen digests.

Thirty-three patients treated with Defibrase for 4–21 for various thrombotic disorders and for anticoagulation in connection with vascular surgery were investigated (IV) Treatment was given in the form of intravenous infusions for one hour 0 01–0 07 ml of Defibrase per kg.b w diluted in 100 ml of physiological saline About half of the patients were only given one infusion per day while the others received two to four infusions per day The maximum dose was 0 154 ml/kg b w./day Patients treated for acute venous thrombosis were given 10 000–12,500 IU of heparin prior to the initial dose of Defibrase in order to achieve immediate anticoagulant effect. Determinations of prothrombin-proconvertin Normotest coagulation factors II V VIII X XII XIII and fibrinogen were performed Plasminogen α_2 macroglobulin FDP concentrations and spontaneous fibrinolytic activity were determined The concentration of antithrombin III was analyzed in some patients Soluble fibrin was demonstrated by the ethanol gelation test and by NH_2 terminal amino acid analysis of fraction I (Abilgaard 1965) of consecutive plasma samples obtained from one patient during the initial 24 hours of Defibrase treatment

The influence of Defibrase on platelet function (III)

No platelet aggregation was found to be induced by the thrombin like enzyme (K 126) at concentrations corresponding to 0 35–1 00 NIH units of thrombin either in normal or in defibrinated PRP These findings are in agreement with those of others (Stocker 1972)

After the Defibrase injections a continuous decrease of the fibrinogen concentration was recorded and in all dogs but one there was a total fibrinogen depletion within three hours. An increased concentration of FDP was found already 30 minutes after the Defibrase injection and a maximum amount (1 024–6 400 mg/ml) was reached within 30 minutes to four hours. Soluble fibrin was detected by the ethanol gelation test till the fibrinogen levels decreased below ⌐ mg/ml

The first experiments performed on anaesthetized dogs showed that after induction of anaesthesia there was an immediate increase of the platelet aggregation which remained for about two hours after which there was a small decrease of the ADP induced aggregation. These changes occurred irrespectively as to whether Defibrase or physiological saline injections were administered. The changes in platelet aggregation were thus regarded to be mediated by the anaesthesia.

In non-anaesthetized dogs a decrease of the ADP induced platelet aggregation was noted At fibrinogen levels below 0.5 mg/ml and at FDP concentrations at or above 0 128 mg/ml the ADP induced platelet aggregation was almost abolished In order to differentiate between the inhibiting effect of low fibrinogen concentrations and high FDP levels fibrinogen was added *in vitro* to PRP samples where the ADP induced aggregation was almost eliminated By restoring the fibrinogen concentration to about 1 8 mg/ml the platelet aggregation was always normalized to pre-experimental level irrespective of the FDP concentration Infusions of FDP to one normal and one defibrinated dog did not influence either ADP or thrombin induced aggregation even when the FDP concentration was at the same level as after Defibrase induced defibrination Kowalski Kopec and Wegrzynowicz (1963) pointed out that the inhibition of ADP induced platelet aggregation by FDP could be overcome by higher ADP concentrations. However Defibrase defibrination a marked decrease of platelet aggrega was observed even at high ADP concentrations. Thus the decreased let aggregation seen during defibrination did not seem to be caused FDP but rather by the decreased fibrinogen concentrations

Bleeding times displayed a gradual increase during the initial four hours of Defibrase induced defibrination. This finding suggests that the bleeding time was influenced by FDP which is in accordance with findings of Kowalski et al (1964) and Cronberg (1968)

Thrombin (1–10 NIH units/ml final concentration) used as aggregating agent proved to induce platelet aggregation even at fibrinogen concentrations below 0.5 mg/ml At these thrombin concentrations the platelet aggregation was reversible and repeated waves of platelet aggregation could be induced by thrombin in the same sample of PRP One interesting observation was that when defibrinated PRP was preexposed to thrombin aggregation could be induced by ADP alone Thrombin induced platelet aggregation is believed to be mediated by a release of ADP from the platelets (Haslam 1964) The fact that platelets do not become refractory to small amounts of ADP (O'Brien 1964)

might explain the repeated responce to thrombin observed in this study Thrombin also releases fibrinogen from the platelets and possibly the local concentration of fibrinogen around the platelets becomes high enough to enable them to respond to ADP exposure

Coagulation studies in patients treated with Defibrase (IV)

The fibrinogen concentration decreased rapidly after infusion of the initial dose of Defibrase (mostly 0 050 ml/kg b w) but there was much variation in the degree of defibrination obtained due to the various pretreatment fibrinogen concentrations The defibrinating effect of the initial dose of Defibrase appeared to last for about 18 hours

Fibrinogen concentrations below 1 0 mg/ml were regarded as adequate since no excessive bleedings were observed postoperatively at this fibrinogen level and no re-occlusions were noted according to the blood flow measurements and clinical observations The initial dose of 0 050 ml/kg b w which was given to most patients generally proved insufficient as a daily dose to maintain this fibrinogen level. Thus the maintenance dose often had to be increased and when it exceeded 0 070 ml/kg b w the Defibrase dosage was divided into two to four infusions per day There was a direct dose responce relationship and there were no signs of resistance to the therapy in any of the patients After termination of the treatment there was an immediate increase of the fibrinogen values Within four days most patients reached 50 % of their initial fibrinogen values Five patients observed for five to eight days after treatment did not return to original fibrinogen concentrations within that time range It was demonstrated that in order to obtain accurate fibrinogen values one had to assure a complete conversion of fibrinogen to fibrin. Methods relying on polymerization rate were often found to give lower values most likely due to interference by FDP

Fibrinaemia (soluble fibrin) was demonstrated by the ethanol gelation test 1—12 hours after the initial dose of Defibrase and occasionally during the continued treatment. Fibrinaemia was also demonstrated by the presence of NH_2 terminal glycine in the clottable portion of Cohn s fraction I obtained from plasma samples taken one to eight hours after the initial Defibrase infusion. Increase of NH_2-terminal glycine in the nonclottable portion of fraction I was furthermore demonstrated This might possibly be due to appearence of clottable high molecular weight FDP e.g Y fragments.

FDP was demonstrated by the haemagglutination inhibition test (Merskey Kleiner and Johnson 1966) and shown to be present throughout the Defibrase treatment mostly with maximum concentration during the initial 24 hours However fibrinolytic activity in plasma samples could never be demonstrated on fibrin plates (Ygge 1970) *In vitro* Defibrase has been shown to be capable of partial degradation of isolated fragments of fibrinogen (Hessel and Blombäck 1971 Mattock and Esnouf 1971) Such a degradation of fibrinogen *in vivo* is however less likely due to the low enzyme concentrations.

A decrease of the plasminogen concentration determined by the radial immunodiffusion technique (Mancini Carbonara and Heremans 1965) was found in all the patients investigated The concentration of plasminogen decreased during the first 24 hours of treatment and thereafter remained at a fairly constant level of about 60 % of the initial plasminogen concentration. There was a good correlation between the amount of fibrinogen removed during the initial 24 hours of treatment and the degree of plasminogen depletion. A correlation was also found between the degree of defibrination and the degree of plasminogen depletion during steady state of treatment

The concentration of α_2 macroglobulin was determined by the Mancini technique in 14 patients during Defibrase treatment. A decrease though not significant, of the α_2-macroglobulin concentration ..s demonstrated However a correlation between the plasminogen .. the α_2-macroglobulin decrease was found

.. observations the plasminogen and α_2-macroglobulin decrease, ..ggest that the fibrinolytic process obtained secondary to the Defibrase induced defibrination is mediated by an activation of plasminogen. The decrease of α_2 macroglobulin should thus be due to a complex formation with plasmin possibly resulting in a more rapid catabolism.

The concentrations of coagulation factors II V VIII X and XII appeared to be unaffected by the Defibrase treatment Proconvertin-prothrombin test and Normotest were also unchanged during the treatment (Dicumarol treatment was instituted two to four days before cessation of Defibrase treatment and affected factors II and X, Normotest and proconvertin-prothrombin tests by the end of the Defibrase treatment.)

On the contrary to other coagulation factors (apart from fibrinogen) the factor XIII concentration, determined by Lorand s fluorescence amino incorporation method (McDonagh, McDonagh and Duckert

1971) decreased significantly in all three patients in whom determinations were made The decrease appeared within 24–48 hours and thereafter a steady state at 50–60 % of the initial level was obtained in two patients. In the third patient a continuous decrease to 25 % of the initial value was observed An activation of factor XIII by Defibrase has been demonstrated *in vitro* (Barlow Holleman and Lorand 1970 McDonagh and McDonagh 1973) and these findings indicate that an activation leading to an increased catabolism of factor XIII also occurs in vivo

The concentration of antithrombin III was unaffected by the Defibrase treatment. In one patient who had previously been treated with heparin an increase of the antithrombin III concentration was observed during Defibrase treatment which might possibly be a result of an increased consumption of antithrombin III during heparin treatment (Blombäck et al 1963)

None of the patients treated developed resistance to Defibrase therapy Such a resistance has however been experimentally induced in dogs (Barlow et al. 1973) In order to investigate if our Defibrase treated patients had developed Defibrase neutralizing antibodies the Defibrase neutralizing capacity of serums from eight patients was tested. None of the serums obtained at the end of Defibrase treatment or obtained five to fourteen months after treatment showed an increased neutralizing capacity In one patient nausea and vomiting occurred in connection with a Defibrase infusion The treatment was discontinued as an allergic reaction could not be ruled out. Serum samples from this patient showed, however no increased Defibrase neutralizing capacity

Summary and conclusion

It was demonstrated that Defibrase *per se* did not induce platelet aggregation

Platelet aggregation induced by ADP and thrombin was determined on successive samples obtained during the initial five hours of Defibrase-induced defibrination in dogs. The ADP-induced platelet aggregation proved to be almost abolished at fibrinogen concentrations below 0.50 mg/ml and FDP concentrations above 0 128 mg/ml. As aggregation could be normalized by reconstituting the f" concentration to 1.80 mg/ml in all samples in which the ADP

FDP was demonstrated by the haemagglutination inhibition test (Merskey Kleiner and Johnson 1966) and shown to be present throughout the Defibrase treatment, mostly with maximum concentration during the initial 24 hours However fibrinolytic activity in plasma samples could never be demonstrated on fibrin plates (Ygge 1970) *In vitro* Defibrase has been shown to be capable of partial degradation of isolated fragments of fibrinogen (Hessel and Blombäck 1971 Mattock and Esnouf 1971) Such a degradation of fibrinogen *in vivo* is however less likely due to the low enzyme concentrations.

A decrease of the plasminogen concentration determined by the radial immunodiffusion technique (Mancini Carbonara and Heremans 1965) was found in all the patients investigated. The concentration of plasminogen decreased during the first 24 hours of treatment and thereafter remained at a fairly constant level of about 60 % of the initial plasminogen concentration. There was a good correlation between the amount of fibrinogen removed during the initial 24 hours of treatment and the degree of plasminogen depletion. A correlation was also found between the degree of defibrination and the degree of plasminogen depletion during steady state of treatment

The concentration of α_2 macroglobulin was determined by the Mancini technique in 14 patients during Defibrase treatment. A decrease though not significant, of the α_2 macroglobulin concentration was demonstrated However a correlation between the plasminogen the α_2-macroglobulin decrease was found

observations the plasminogen and α_2-macroglobulin decrease, gest that the fibrinolytic process obtained secondary to the Defibrase induced defibrination is mediated by an activation of plasminogen. The decrease of α_2 macroglobulin should thus be due to a complex formation with plasmin possibly resulting in a more rapid catabolism.

The concentrations of coagulation factors II V VIII X and XII appeared to be unaffected by the Defibrase treatment Proconvertin-prothrombin test and Normotest were also unchanged during the treatment. (Dicumarol treatment was instituted two to four days before cessation of Defibrase treatment and affected factors II and X, Normotest and proconvertin prothrombin tests by the end of the Defibrase treatment)

On the contrary to other coagulation factors (apart from fibrinogen) the factor XIII concentration determined by Lorand's fluorescence amino incorporation method (McDonagh, McDonagh and Duckert

ON THE METABOLISM OF THE THROMBIN-LIKE ENZYME FROM BOTHROPS ATROX

Previous investigations

Purified α_2 macroglobulin was by Lanchantin et al (1966) and Abildgaard (1969) reported to progressively inhibit thrombin It has also been shown that the inactivation resulted from complex formation between α_2-macroglobulin and thrombin (Shulman 1954 Lanchantin (1966) Furthermore it was shown that the proteolytic activity of thrombin was inhibited while its esterolytic activity on small synthetic esters was mainly unaffected suggesting that the »active site» was not completely blocked by the association to α_2 macroglobulin (Lanchantin et al. 1966 James Taylor and Fudenberg 1967)

The coagulant activity of the purified thrombin like enzyme from the venom of Agkistrodon rhodostoma Arvin, was shown to progressively decrease when incubated with normal human serum (Pitney and Regoeczi 1970) The inhibition was shown to result from an association of Arvin to α_2 macroglobulin and possibly also to anti thrombin III.

Present investigations

The main purpose of these studies was to investigate whether Defibrase interacted with serum proteins *in vitro* and *in vivo* and whether physiological inhibitors of proteolytic enzymes were capable of blocking the enzymatic activity of Defibrase Secondly a prelim mary study of the elimination of the thrombin like enzyme and the urinary excretion of the labelled material was performed in dogs.

For these studies a highly purified lyophilized preparation of the thrombin-like enzyme (DG 144) with a thrombin-like activity equiva lent to about 100 NIH units of thrombin per mg was labelled with ^{125}I-isotope according to two different methods described by Hunter and Greenwood (1962) and by McFarlane (1963) The former labelled enzyme (in text denoted type A) had a specific activity of about 45

platelet aggregation was almost abolished, it was concluded that the low fibrinogen concentration was of major importance for the decrease of platelet aggregation This conclusion was also supported by the fact that on infusions of FDP no effect on the platelet aggregation was obtained with the doses of ADP used in this study On the other hand the bleeding time increased continuously during the defibrination period indicating that it might be affected by the appearance of FDP as well as by the low fibrinogen concentration

Thrombin on the other hand appeared to induce platelet aggregation regardless of the FDP or fibrinogen concentrations After pre-exposure of defibrinated PRP to thrombin platelet aggregation could be induced by ADP alone. This finding might be explained by the fact that thrombin releases fibrinogen from the platelet and thereby enables ADP to induce aggregation.

In Defibrase treated patients it was demonstrated that the maintenance dose of Defibrase necessary to keep the fibrinogen concentration below 1 00 mg/ml was in the range of 0 050–0 150 ml of Defibrase per kg b w /day Secondary to the defibrination, an increased fibrinolytic activity seemed to be obtained as judged from the appearance of FDP although no increased fibrinolytic activity could be demonstrated on fibrin plates. A decrease of the plasminogen concentration correlated to a small decrease of the α_2-macroglobulin concentration was also demonstrated The latter findings indicate that the fibrin (-ogen) degradation was produced by digestion with plasmin.

Fibrinaemia was demonstrated by the ethanol gelation test and by NH_2 terminal analysis during the initial phase of defibrination.

No signs of generalized dissiminated intravascular coagulation were found in terms of consumption of coagulation factors II V VIII and X.

Antithrombin III was also unaffected during Defibrase treatment.

Factor XIII decreased to about 50 % of the initial value during Defibrase treatment This is in accordance with earlier *in vitro* findings where Defibrase proved to activate factor XIII

As judged from the response to treatment with regard to fibrinogen values no patient was resistant to therapy The fact that the doses of Defibrase had to be increased during treatment might be explained solely by an increased fibrinogen synthesis during Defibrase treatment. An allergic reaction cannot be excluded in one patient. However serum samples from this patient and other patients did not show an increased Defibrase neutralizing capacity *in vitro*

ON THE METABOLISM OF THE THROMBIN-LIKE ENZYME FROM BOTHROPS ATROX

Previous investigations

Purified α_2 macroglobulin was by Lanchantin et al. (1966) and Abildgaard (1969) reported to progressively inhibit thrombin It has also been shown that the inactivation resulted from complex formation between α_2 macroglobulin and thrombin (Shulman 1954 Lanchantin (1966) Furthermore it was shown that the proteolytic activity of thrombin was inhibited while its esterolytic activity on small synthetic esters was mainly unaffected suggesting that the »active sites was not completely blocked by the association to α_2-macroglobulin (Lanchantin et al. 1966 James Taylor and Fudenberg 1967)

The coagulant activity of the purified thrombin like enzyme from the venom of Agkistrodon rhodostoma Arvin was shown to progressively decrease when incubated with normal human serum (Pitney and Regoeczi 1970) The inhibition was shown to result from an association of Arvin to α_2 macroglobulin and possibly also to antithrombin III

Present investigations

The main purpose of these studies was to investigate whether Defibrase interacted with serum proteins *in vitro* and *in vivo* and whether physiological inhibitors of proteolytic enzymes were capable of blocking the enzymatic activity of Defibrase Secondly a preliminary study of the elimination of the thrombin like enzyme and the urinary excretion of the labelled material was performed in dogs

For these studies a highly purified lyophilized preparation of the thrombin-like enzyme (DG 144) with a thrombin-like activity equivalent to about 100 NIH units of thrombin per mg was labelled with ^{125}I-isotope according to two different methods described by Hunter and Greenwood (1962) and by McFarlane (1963) The former labelle enzyme (in text denoted type A) had a specific activity of abou

platelet aggregation was almost abolished, it was concluded that the *low fibrinogen* concentration was of major importance for the decrease of platelet aggregation This conclusion was also supported by the fact that on infusions of FDP no effect on the platelet aggregation was obtained with the doses of ADP used in this study On the other hand the bleeding time increased continuously during the defibrination period indicating that it might be affected by the appearance of FDP as well as by the low fibrinogen concentration.

Thrombin on the other hand appeared to induce platelet aggregation regardless of the FDP or fibrinogen concentrations. After pre-exposure of defibrinated PRP to thrombin platelet aggregation could be induced by ADP alone This finding might be explained by the fact that thrombin releases fibrinogen from the platelet and thereby enables ADP to induce aggregation

In Defibrase treated patients it was demonstrated that the maintenance dose of Defibrase necessary to keep the fibrinogen concentration below 1 00 mg/ml was in the range of 0 050--0 150 ml of Defibrase per kg b w /day Secondary to the defibrination, an increased fibrinolytic activity seemed to be obtained as judged from the appearance of FDP although no increased fibrinolytic activity could be demonstrated on fibrin plates A decrease of the plasminogen concentration correlated to a small decrease of the a_2 macroglobulin concentration was also demonstrated. The latter findings indicate that the fibrin (-ogen) degradation was produced by digestion with plasmin.

Fibrinaemia was demonstrated by the ethanol gelation test and by NH_2 terminal analysis during the initial phase of defibrination

No signs of generalized disseminated intravascular coagulation were found in terms of consumption of coagulation factors II V VIII and X.

Antithrombin III was also unaffected during Defibrase treatment.

Factor XIII decreased to about 50 % of the initial value during Defibrase treatment This is in accordance with earlier *in vitro* findings where Defibrase proved to activate factor XIII

As judged from the response to treatment with regard to fibrinogen values no patient was resistant to therapy The fact that the doses of Defibrase had to be increased during treatment might be explained solely by an increased fibrinogen synthesis during Defibrase treatment. An allergic reaction cannot be excluded in one patient However serum samples from this patient and other patients did not show an increased Defibrase neutralizing capacity *in vitro*

Experimental conditions

Due to difficulties in obtaining large quantities of homogeneous fragment E from the serum of patients treated with Defibrase plasmic digestion of human fibrinogen (fraction I–4) was performed in vitro and fragment E was isolated according to the method described by Gårdlund et al (1972)

Fragment E was subsequently subjected to cleavage by cyanogen bromide (CNBr) as described by Blombäck et al (1972) The fragments obtained were separated on Sephadex G 100 in 10% acetic acid The main fragment obtained after cleavage with CNBr was further subjected to tryptic digestion and the tryptic peptides were separated by means of two dimensional low voltage electrophoresis – chromatography ("peptide mapping") on thin-layer cellulose plates as described by Blombäck et al (1968) The peptides of the ninhydrin positive spots were eluted for amino acid analysis (Blombäck et al. 1972) The amino acid compositions of the eluted peptides were thereafter compared with those of tryptic peptides of N DSK at corresponding positions on the peptide map

Immunological studies were also performed by means of immuno-diffusion (Ouchterlony 1949) and immuno-electrophoresis (Scheidegger 1955)

Chemical and immunological relation between fragment E and N DSK

Immuno-electrophoretic and immunodiffusion studies revealed a partial identity between fragment E and N DSK. It was furthermore shown by immuno-diffusion studies using antisera against isolated chains of N DSK, that parts of the Aα and Bα chains of N-DSK were present in fragment E

Release of fibrinopeptide A (Blombäck et al. 1966) from fragment E was demonstrated Since N DSK is obtained by cleavage of methionyl bonds in fibrinogen by CNBr and fragment E contained 3–4 methionine residues per mole it was decided to treat fragment E with CNBr and isolate the fragments obtained On Sephadex G 100 three peaks of UV absorbing material was obtained The first and largest peak was found to contain material giving a precipitin reaction with anti-N DSK serum, showing complete immunological identity with N-DSK. The fragment located in the first peak had a molecular

consisted of three popypeptide chains with molecular weights of 5,500 6,500 and 8 800 respectively as determined by polyacrylamide gel electrophoresis in SDS (McDonagh et al. 1972) This fragment also proved to have almost the same carbohydrate (Hewitt 1937) and disulphide content (Zahler and Cleland 1968) as fragment E and N DSK During thrombic digestion fibrinopeptide A was released from this fragment Due to the great similarities between N DSK and this large component of fragment E it was denoted »E-knot»

Immunological identity was demonstrated between »E-knot» and N DSK and partial identity between »E-knot» and fragment E

Quantitative NH_2 terminal analysis (Blombäck et al 1972 Blombäck et al 1973) of fragment E and »E-knot» were identical. Almost two moles of tyrosine were obtained per mole of »E knot suggesting that the NH_2 terminal part of the γ chain was unaffected by plasmic digestion Alanine was determined to 1.8 moles per mole indicating the presence of fibrinopeptide A. Lysine was the third main NH_2 terminal amino acid 0.9 moles per mole probably representing the NH_2 terminal amino acid of the Bβ chain Appreciable amount of aspartic acid glycine and valine were also found suggesting a heterogeneity of fragment E

Peptide mapping of E-knot» showed that all spots of »E-knot» could be accounted for by spots in N DSK Amino acid compositions of the ιι eluted from the peptide map of »E-knot» were found to be in agreement with the known amino acid sequences of tryptic peptides from N-DSK at corresponding positions on the peptide map The studies suggested the following main structure for »E-knot» (Aα 1 Ala – 51Met β 54Lys – 115Met, γ 1Tyr – 58Lys$)_2$ The yields of some peptides were however found to be lower than the average yield indicating an incomplete plasmic cleavage resulting in a heterogeneity Fibrinopeptide A was thus found to be missing in about 40 % of the E-knot» molecules The COOH-terminal, 51Met, of the Aα chain was also missing to the same extent. Low yields of the tryptic peptides Bβ 94 Asn – 115 Met and γ 54 Tyr – 58 Lys suggested heterogeneity even in these parts of the fragment

The fact that the spot representing the amino acid residue Aα 51 Met of E-knot» was not present after tryptic digestion of intact fragment E indicates that the Aα chain of fragment E extends outside »E-knot» A difference in the mobilities of the β chains from »E-knot» and fragment E on polyacrylamide gel electrophoresis suggested that the β chain of fragment E extends beyond 115Met of the E-knot» Two

additional peaks of UV absorbing material were obtained by gel filtration of CNBr treated fragment E The materials of the second and third peaks were found to have molecular weights of about 4 000–5 000 and 500–1 000 and might represent the peptides extending outside »E-knot» in the Aα and β chains.

Summary and conclusion

After cleavage of fragment E with cyanogen bromide a fragment having a molecular weight of 43 000 and consisting of three polypeptide chains was isolated »E knot» It was shown that the E-knot contained fibrinopeptide A and the same amount of disulphide bridges and neutral sugars as fragment E and the NH_2 terminal disulphide knot (N-DSK) Furthermore E-knot» showed immunological identity with N DSK. By two dimensional thin layer electrophoresis – chromatography tryptic peptides of »E-knot» were separated. These peptides were subsequently eluted subjected to amino acid analysis and the amino acid compositions compared with the known sequences of tryptic peptides from N DSK at corresponding positions on the peptide map

The results suggested the following formula for »E-knot» (Aα 1Ala – 51Met, β 54Lys – 115Met γ 1Tyr – 58Lys)$_2$ Heterogeneity was demonstrated for peptides Aα 1Ala – 16Arg and 51Met, β 94[illegible] 115Met and γ 54Tyr – 58Lys. Sequences extending beyond Aα 51Met and β 115Met are most likely present in fragment E as those chains of fragment E appeared to be cleaved by CNBr at these positions.

GENERAL SUMMARY

The in vivo effects of the thrombin like enzyme from Bothrops atrox was *investigated in dogs and in humans Intravenous administration of* similar doses of the enzyme produced a rapid depletion of the circulating fibrinogen and a secondary fibrinolytic process in dogs as well as in man No thromboembolic complications were observed. Histological examinations of dog organs revealed that during the early phase of the defibrination sometimes minor capillary fibrin deposits were produced However these deposits did not seem to interfere with the circulation. Only small amounts of fibrinogen related material were found in the liver and in the spleen of some dogs indicating a minor importance of the reticuloendothelial system for the removal of fibrin.

No spontaneous bleeding symptoms occurred during the defibrination or in the defibrinated state. A marked decrease of the platelet aggregation was, however demonstrated in dogs during the early phase of defibrination most likely caused by the low fibrinogen concentration not by the increased amounts of fibrinogen degradation products -) Bleeding times also increased in dogs during this period and to be affected by the increased amount of FDP In humans t defibrinated for several days because of thrombotic diseases coagulation factors II V VIII X and XII were unaffected by the treatment. Factor XIII on the other hand decreased by 40–75 % indicating an activation by the enzyme

The secondary fibrinolytic process was in dogs found to be delayed but not inhibited by simultaneous administration of fibrinolytic inhibitors. The fibrinolysis caused an increased amount of FDP in the circulation both in dog and man and in humans the maximum concentrations mostly occurred during the initial 24 hours of treat ment. The secondary fibrinolytic process caused in humans a decrease of the plasminogen concentration to about 50 % of the initial value

The thrombin-like enzyme was shown to associate with α_2 macroglobulin *in vitro* and most probably *in vivo* The complex formed was devoid of clot promoting activity but was able to hydrolyze a synthetic tripeptide substrate. The importance of the α_2-macroglobulin enzyme complex *in vivo* is obscure The elimination of ^{125}I-labelled enzyme

was rapid and only about 2 % of the initial radioactivity remained in the circulation after 24 hours.

The primary structure of the plasmic degradation product of human fibrinogen fragment E (m.w 50 000) was investigated By treatment of framgment E with cyanogenbromide (CNBr) one large fragment (m.w 45,000) showing complete immunological identity with the NH_2 terminal disulphide knot (N DSK) was produced. It was shown that this large fragment, produced by CNBr treatment was a structure included in N DSK with the formula (Aα 1Ala – 51Met, β 54Lys – 115Met γ 1Tyr – 58Lys)$_2$

ACKNOWLEDGEMENTS

Professor Birger Blombäck and Assistant Professor Margareta Blombäck were my stimulating teachers in coagulation research to whom I am greatly indepted for never failing interest and support for valuable guidance and constructive criticism as well as for generously placing facilities at my disposal throughout this work

I also wish to express my sincere gratitude to

Assistant Professor Per Olsson for his deep interest in this work and for valuable help and inspiring discussions.

Assistant Professor Sam Nordström who introduced me into animal experimental research for friendly cooperation valuable and stimulating discussions

Professor Arne Ljungqvist for stimulating guidance positiv criticism and cooperation

Professor Sune Larsson and Professor Lars Hammarström for valuable help and guidance with the autoradiographic studies as well as for facilities for this work at my disposal

Hans Johnson for friendly and valuable help and for all stimulating discussions.

Dr Bengt Gårdlund and Dr Barbara Kowalska Loth for all help and the inspiring cooperation.

Dr Birgit Hessel for good help and guidance in the biochemistry work and interesting discussions.

Assistant Professor Stig Arne Johansson for his friendly cooperation and constructive criticism.

Professor Erik Berglund former Head of the Thoracic Surgery Research Laboratory and Professor Dag Hallberg present Head of the same laboratory for generously providing facilities required for the animal experiments at my disposal

Furthermore I wish to express my graditude to the laboratory assistants at the Coagulation Laboratory who performed all the coagulation analysis and in many ways have facilitated my work, notably Mrss. M Apostolidis H Bergström R Flagell C Hagström E Holmberg C Lind A K. Pernholm E Sandahl, M. Tingeborn U Wallinder and U B Wikström. The staff of the Department of Blood

Coagulation Research for skilled and friendly assistance in the chemical work notably Mrss. E Andersson H Messel C Netré S Söderman and L. Therkildsen and Engineers J Reuterby and W Finkbeiner The staff of the Thoracic Surgery Research Laboratory for valuable help in the animal experiments notably Mrss U Engqvist K. Librén and M Wohlin and Mr L. Henriksson.

Mrss. T Esh S Sigfridsson, E Sörensen and Miss U Hoffmann and L. Wikbladh for excellent secreterial help

Mr Ivor Perschke for correcting the English of this manuscript.

This work was supported by grants from the Swedish Medical Research Council, No 13X 2475 and No 19X 520 National Institute of Health, No HL07379 Swedish Association for Medical Research and T Nilson s Research Fund

BLOMBÄCK, B., M. BLOMBÄCK and E. HOLMBERG A new method for fractionation of proteins. *Acta chem. scand.* 1966 *20* 2317–2319

BLOMBÄCK, B. M. BLOMBÄCK H. LAGERGREN and P OLSSON The heparin co-factor activity in plasma and its relation to the anticoagulating effect of intravenously injected heparin. *Acta physiol. scand* 1963 *58* 306–318

BLOMBÄCK, B., M BLOMBÄCK and I.M NILSSON Coagulation studies on Reptilase» an extract of the venom from Bothrops jararaca. *Thrombos. Diathes haemorrh.* 1957 *1* 76–86

BLOMBÄCK, B., N.J GRÖNDAHL, B. HESSEL, S. IWANAGA and P WALLEN Primary structure of fibrinogen and fibrin II. Structural studies on NH_2-terminal part of Ychain. *J Biol. Chem.* 1973 *248* 5806–5870

BLOMBÄCK, B., B. HESSEL, S IWANAGA J REUTERBY and M. BLOMBÄCK, Primary structure of human fibrinogen and fibrin I Cleavage of fibrinogen with cyanogen bromide Isolation and characterization of NH_2-terminal fragments of the α (A») chain. *J Biol. Chem.* 1972 *247* 1496–1512

BLOMBÄCK, B and T.C. LAURENT N-terminal and light-scattering studies on fibrinogen and its transformation to fibrin. *Arkiv Kemi.* 1958. *12* 137–146

BRUCK, H., A method for the examination of hemostatics by surgical test and clinical records of the use of Reptilase» on a great many patients. *Wien. klin. Wschr* 1957 *69* 571–574

BRUCK, H. and G SALEM Reptilase ein Hämostatikum zur Prophylaxe und Therapie bei chirurgischen Eingriffen. *Wien klin. Wschr* 1954. *66* 395–397

CHAN K.E., The hypofibrinogenaemic state in Malayan viper poisoning 1964. Ph.D Thesis. Queen s Coll. St. Andrew's University

CHAN K.E. and H.A. REID Fibrinolysis and the defibrination syndrome of Malayan viper bite *Lancet.* 1964 *1* 461–463

CHAN K.E., C.R. RIZZA and M.P HENDERSON A study of the coagulant properties of Malayan pit-viper venom. *Brit. J Haemat.* 1965 *11* 646–653

COPLEY A.L., S BANERJEE and A. DEVI, Studies on snake venoms on blood coagulation I. The thromboserpentin (thrombin-like) enzyme in the venoms. *Thrombos. Res.* 1973 2 487–508.

CRONBERG S., Effect of fibrinolysis on adhesion and aggregation of human platelets. *Thrombos Diathes. haemorrh.* 1968. *19* 474–482.

CROSS M.J., Effect of fibrinogen on the aggregation of platelets by adenosin diphosphate *Thrombos. Diathes. haemorrh.* 1964 *12* 524–527

DEUTSCH, E., Blutgerinnungsfactoren. Franz Deuticke Wien. 1955 208.

DURANTE, L.J., A. MOUTSOS R.B AMBROSE W Y DUNCAN W FLEMING H.H. ZINSSER and L.L. PHILLIPS Postprostatectomy bleeding *A n. Surg* 1962 *156.* 781–792

EAGLE H., The coagulation of blood by snake venoms and its physiologic significance. *J.Exp Med.* 1937 *65* 613–639

ESNOUF M.P and R. MARSHALL, The effect of blockade of th reticuloend othelial system and of hypotension on the response of dogs to Ancistrodon rhodostoma venom. *Clin. Sci.* 1968 *35* 261–272

ESNOUF M.P and G.W TUNNAH The isolation and properties thrombin-like activity from Ancistrodon rhodostoma venom. *Bri*
1967 *13* 581–590

FLEISCHHACKER H., Zur Therapie der Gerinnungsstörungen. *Wien med. Wschr* 1954 *104* 171–173

GLANINGER, J Über die Verwendung des Hemostyptikums Reptilase in der Hals- Nasen- und Ohrenheilkunde *Mschr Ohrenheilk.* 1955 *89* 63–66

GROSS J., R BOGOROCH N.J NADLER and C.P LEBLOND The theory and methods of the radioautographic localization of radioelements in tissues. *Am. J Roentgenol.* 1951 *65* 420–458

GARDLUND B B. KOWALSKA LOTH N.J GRÖNDAHL and B. BLOMBÄCK Plasmic degradation products of human fibrinogen I Isolation and characterization of Fragments E and D and their relation to disulfide knots *Thrombos. Res.* 1972 *1* 371–388

HASLAM R.J., Role of adenosin diphosphate in the aggregation of human blood – platelets by thrombin and by fatty acids *Nature* 1964 *202.* 765–768

HEISS H., Ein Beitrag zur hämostatischen Therapie beim Kollumkarzinom. *Wien. med. Wschr* 1954 *104* 833–834

HESSEL B and M BLOMBÄCK, The proteolytic action of the snake venom enzymes Arvin and Reptilase on N-terminal chain-fragments of human fibrinogen *FEBS Letters.* 1971 *18* 318–320

HEWITT L.F., Separation of serum albumin into two fractions II Observations on the nature of the glycoprotein fraction. *Biochem. J* 1937 *31* 360–366

HOHNEN H W., Experimentelle Studien zur Frage der Beeinflussung der Blutgerinnung durch Reptilase *Z. Ges. Exp Med.* 1957 *128* 427–436

HIRSCH J.A., P FLETCHER and S SHERRY Effect of fibrin and fibrinogen proteolysis products on clot physical properties. *Am. J Physiol.* 1965 *209* 415–424

HUNTER, W.M and F C. GREENWOOD Preparation of iodine–131 labelled human growth hormone of high specific activity *Nature* 1962 *194* 495–496

K., F B. TAYLOR, Jr and H.H. FUDENBERG The effect of α_2-macroglobulin in human serum on trypsin, plasmin and thrombin activities. *Biochem. Biophys. Acta.* 1967 *133* 374–376

von KLOBUSITZKY D Biochemische Studien über die Gifte der Schlangengattung Bothrops I *Naunyn Schmiedeberg's Arch. exp Path. Pharmak* 1935 *179* 204–216

von KLOBUSITZKY D. and P KÖNIG Biochemische Studien über die Gifte der Schlangengattung Bothrops III *Naunyn-Schmiedeberg's Arch. exp Path. Pharmak.* 1936 *181* 387–398

KOWALSKI E., A.Z. BUDZYNSKI M KOPEC, Z.S. LATALLO B. LIPINSKI and Z. WEGRZYNOWICZ Studies on the molecular pathology and pathogenesis of bleeding in severe fibrinolytic states in dogs. *Thrombos. Diathes. haemorrh.* 1964 *12.* 69–86

KOWALSKI E., M. KOPEC and Z. WEGRZYNOWICZ, Influence of fibrinogen degradation products (FDP) on platelet aggregation, adhesiveness and viscous metamorphosis. *Thrombos. Diathes haemorrh.* 1963 *10* 406–423

KWAAN H.C. and G.H. BARLOW The mechanism of action of Arvin and Reptilase *Thrombos. Diathes. haemorrh.* 1971 Suppl. 47 361–369

LANCHANTIN G F., M.L. PLESSET J.A. FRIEDMANN and D.W HART Dissociation of esterolytic and clotting activities of thrombin by trypsin-binding macroglobulin. *Proc So xp Biol. Med. (N Y)* 1966 *121* 444–449

LARRIEU M.J., Discussion. *Scand J Haemat.* 1971 Suppl. *13* 65–66

LARRIEU M.J., S INCEMAN and V MARDER, Action des produits de dégradation du fibrinogén sur les fonctions plaquettaires. *Nouvelle Rev Franc. Hématol.* 1967 *7* 691–704

LAURENT T C and B BLOMBÄCK, On the significance of the release of two different peptides from fibrinogen during clotting *Acta chem. scand.* 1958 *12* 1875–1877

LEE L., Reticuloendothelial clearance of circulating fibrin in the pathogenesis of the generalized schwartzman reaction. *J Exp Med* 1962 *115* 1065–1082

LORAND L., Personal communication. 1972

MANCINI G., A.O CARBONARA and J.F HEREMANS Immunochemical quantitation of antigens by single radial immunodiffusion. *Int J Immunochem.* 1965 2. 235–254

MARDER, V.J., Identification and purification of fibrinogen degradation products produced by plasmin Considerations on the structure of fibrinogen. *Scand. J Haemat.* 1971 Suppl. *13* 21–36.

MARDER, V.J., A.Z. BUDZYNSKI and H.L. JAMES High molecular weight derivates of human fibrinogen produced by plasmin III Their NH_2 terminal amino acids and comparison with the NH_2 terminal disulphide knots *J Biol. Chem.* 1972 *247* 4775–4781

MARCHALL, R. and M.P ESNOUF The effect of Ancistrodon rhodostoma venom in the dog *Clin. Sci.* 1968 *35* 251–259

MATTOCK, P and M.P ESNOUF Differences in the subunit structure of human fibrin formed by the action of Arvin Reptilase and thrombin. *Nature (New Biol.)* 1971 *233* 277–279

McDONAGH, J and R.P McDONAGH Activation of human plasma factor XIII IVth internat. congr thrombos. haemostas., Vienna. Abstract vol. 1973 189

McDONAGH Jr R.P., J McDONAGH and F DUCKERT The influence of fibrin crosslinking on the kinetics of urokinase induced clot lysis. *Brit. J Haemat* 1971 *21* 323–332

McDONAGH, J., H. MESSEL, R.P McDONAGH, Jr., G MURANO and B. BLOMBÄCK, Molecular weight analysis of fibrinogen and fibrin chains by an improved sodium dodecyl sulfate gel electrophoresis method. *Biochim Biophys. Acta.* 1972 *257* 135–142

McFARLANE A.S., In vivo behavior of [131]I-fibrinogen. *J Clin. Invest.* 1963 *42* 346–361

MELLANBY J., The coagulation of blood. Part II. The action of snake venoms, peptone and leech extract. *J Physl l.* 1909 *38* 441–503

MERSKEY C., G.J KLEINER and A.J JOHNSON Quantitative estimation of split products of fibrinogen in human serum, relation to diagnosis and treatment. *Blood.* 1966. 28. 1–18

MILLS D.A., A molecular model f the proteolysis of human fibrinogen by plasmin. *Biochim. Biophys. Acta.* 1972 *263* 619–626

MILLS D.A. and D C. TRIANTAPHYLLOPOULOS Distribution of among the polypeptide chains and plasmin digest products of human *Arch. Biochem. Biophys.* 1969 *28.* 135–142.

NAHAS L., K.W E. DENSON and R.G MACFARLANE, A study of the coagulant action of eight snake venoms. *Thrombos. Diathes. haemorrh.* 1964 *12* 355–367

NORDSTRÖM S. and E. ZETTERQVIST Effects of thrombin infusions upon [131]I-labelled fibrinogen in dogs. *Acta physiol. scand.* 1968. *72*. 85–99

NORDSTRÖM S and E. ZETTERQVIST Effects of epsilon aminocaproic acid (EACA) and Trasylol on thromboplastin induced intravascular coagulation, studied in dogs with iodine labelled fibrinogen. *Acta physiol. scand.* 1969 *76* 95–105

NORMAN P.S., Antiplasmins. *Fed Proc* 1966 *25* 63–67

NUSSENZWEIG V., M SELIGMANN J PELMONT and P GRABAR, Les produits de dégradation du fibrinogène humain par la plasmine I Séparation et propriétés physico-chemiques. *Ann. Inst. Pasteur* 1961 *100*. 377–389

OHLENSCHLÄGER G. and J SCHWALBE Klinische Erfahrungen mit Reptilase bei haemorrhagischen Diathesen und postoperative Blutungen. *Med. Mschr* 1966. *20* 179–181

O'BRIEN J R., A comparison of platelet aggregation produced by seven compounds and a comparison of their inhibitors. *J clin. path.* 1964 *17* 275–281

OUCHTERLONY Ö Antigen-antibody reactions in gels II. Factors determining the site of the precipitate *Arkiv Kemi* 1949 *43* 43–48.

PITNEY W R. and E REGOECZI Inactivation of Arvin by plasma proteins. *Brit. J Haemat* 1970 *19* 67–81

PRENTICE C.R.M., A.A. HASSANEIN A.G G. TURPIE G.P McNICOL and A.S DOUGLAS Changes in platelet behaviour during Arvin therapy *Lancet.* 1969 *1* 644–647

E. and W.R. BELL, In vivo behaviour of the coagulant enzyme from Agkistodon rhodostoma venom Studies using [131]I Arvin. *Brit. J Haemat.* 1969 *16* 573–587

REGOECZI E., J GERGELY and A.S McFARLANE, In vivo effects of Agkistrodon rhodostoma venom: studies with fibrinogen [131]I. *J clin. Invest.* 1966. *45* 1202–1212

REID H.A. E-aminocaproic acid and fibrinolysis in viper-bite defibrination. *Lancet.* 1965 *2* 5–7

REID H.A., K.E. CHAN and P.C. THEAN Prolonged coagulation defect (defibrination syndrome) in Malayan viper bite. *Lancet.* 1963 *1* 621–626

RIMON A., Y SHAMASH and B. SHAPIRO The plasmin inhibitor of plasma IV Its action on plasmin chymotrypcin and thrombin. *J Biol. Chem.* 1966. *241* 5102–5107

SHARP A.A. B.A. WARREN A.M PAXTON and M.J ALLINGTON Anticoagulant therapy with a purified fraction of Malayan pit viper venom. *Lancet.* 1968 *1* 493–499

SHEIDEGGER, J.J Une micro-méthode de l'immuno-électrophorèse. *Int. Arch. Allergy* 1955 7 103–110

SHULMAN N.R. A proteolytic inhibitor with anticoagulant activity separated from human urine and plasma. *J Biol. Chem.* 1954. *213* 655–671

SILBERMAN S., E.V POTTER and H.C. KWAAN Effects of Arvin in Mice. Immunofluorescent and histochemical studies. *Exp. Molec. Path.* 1971 *14* 67–74

SOLUM N.O and H. STORMORKEN Influence of fibrinogen on the aggregation of washed human blood platelets induced by adenosin dihposphate thrombin, collagene and adrenaline. *Scand. J clin. Lab Invest.* 1965 *17* Suppl. 84. 170–182

STACHER, A. and J BÖHNEL, Experimental and clinical research on the hemostatic action of snake venoms. *Wien. klin. Wschr* 1959 *71* 333–335

STEINHOFF H., Das Hämostypicum Reptilase in klinischer Prufung als Prophylacticum und Therapeuticum. *Med. Mschr* 1960 *14* 527–530

STOCKER, K., Personal communication. a 1970 b 1972 c. 1973

STOCKER, K. and G.H BARLOW Protein-chemical characterization of Defibrase Lecture at IVth Angiologisches Symposion. Aggertalklinik, Engelskirchen. 1973

STRAUB P W., On the methabolism of thrombin-like snake venom enzymes in man. Lecture at IVth Angiologisches Symposion Aggertalklinik, Engelskirchen. 1973

SUGANO T and R. MARUYAMA, Experience of the use of Reptilase in the field of otorhinolaryngology *Otolaryngology (Tokyo)* 1964. *36* 365–367

SVENDSEN L., B. BLOMBÄCK M. BLOMBÄCK and P.I. OLSSON Synthetic chromogenic subsrates for determination of trypsin, thrombin and thrombin-like enzymes. *Thrombos Res* 1972 *1* 267–278

TÓTH S., L. KASSAY E BARZÓ and T SZILÁGYI The effect of haemocoagulase (Reptilase) on the local Schwartzman phenomenon. *Acta Microbiol. Acad. Sci. hung* 1967 *14* 371–373

TÓTH S., G. LÉVAI and T SZILÁGYI Effect of Reptilase on thrombo-haemorrhagic reactions. *Acta morphologic Acad. Sci. hung* 1971 *19* 123–130

VERMYLEN C. R.A. de VREKER and M. VERSTRAETE, A rapid enzymatic method for assay of fibrinogen fibrin polymerization time (FPT-test) *Clin. Chim. Acta.* 1963 *8* 418–424

YGGE, J., Studies on blood coagulation and fibrinolysis in conditions associated with an increased indicence of thrombosis. *Scand. J Haemat.* 1970 Suppl. 11

ZAHLER, W.L. and W W CLELAND A specific and sensitive assay for disulfides. *J Biol. Chem.* 1968 *243* 716–719

ZÜRN H., Untersuchungen über Einwirkung verschiedener Schlangengiftpreparate auf die Blutgerinnung *Theoretische Medizin.* 1959 *13* 117–124.

NAHAS L., K.W.E. DENSON and R.G MACFARLANE A study of the coagulant action of eight snake venoms. *Thrombos. Diathes. haemorrh.* 1964 *12.* 335–367

NORDSTRÖM S and E. ZETTERQVIST Effects of thrombin infusions upon ^{131}I-labelled fibrinogen in dogs. *Acta physiol. scand.* 1968 *72* 85–99

NORDSTRÖM, S and E. ZETTERQVIST Effects of epsilon aminocaproic acid (EACA) and Trasylol on thromboplastin-induced intravascular coagulation, studied in dogs with iodine labelled fibrinogen. *Acta physiol. scand.* 1969 *76* 93–105

NORMAN P.S., Antiplasmins. *Fed Proc* 1966 *25* 63–67

NUSSENZWEIG V., M SELIGMANN J PELMONT and P GRABAR Les produits de dégradation du fibrinogène humain par la plasmine I Séparation et propriétés physico-chemiques. *Ann. Inst. Pasteur* 1961 *100* 377–389

OHLENSCHLÄGER, G and J SCHWALBE Klinische Erfahrungen mit Reptilase bei haemorrhagischen Diathesen und postoperative Blutungen. *Med. Mschr* 1966 *20* 179–181

O'BRIEN J.R., A comparison of platelet aggregation produced by seven compounds and a comparison of their inhibitors. *J clin. path.* 1964. *17* 275–281

OUCHTERLONY Ö., Antigen-antibody reactions in gels II. Factors determining the site of the precipitate *Arkiv Kemi.* 1949 *43* 43–48.

PITNEY W.R. and E. REGOECZI Inactivation of Arvin by plasma proteins. *Brit. J Haemat.* 1970 *19* 67–81

PRENTICE C.R.M., A.A. HASSANEIN A.G.G TURPIE G.P McNICOL and A.S DOUGLAS Changes in platelet behaviour during Arvin therapy *Lancet.* 1969 *1* 644–647

[illegible]ECZI E. and W R. BELL, In vivo behaviour of the coagulant enzyme from Agkistodon rhodostoma venom: Studies using ^{131}I Arvin. *Brit. J Haemat.* 1969 *16* 573–587

REGOECZI E., J GERGELY and A.S McFARLANE, In vivo effects of Agkistrodon rhodostoma venom: studies with fibrinogen ^{131}I *J clin. Invest.* 1966. *45* 1202–1212

REID H.A. E-aminocaproic acid and fibrinolysis in viper-bite defibrination. *Lancet.* 1965 2. 5–7

REID H.A. K.E. CHAN and P.C. THEAN Prolonged coagulation defect (defibrination syndrome) in Malayan viper bite. *Lancet.* 1963 *1* 621–626

RIMON A. Y SHAMASH and B. SHAPIRO The plasmin inhibitor of plasma IV Its action on plasmin chymotrypcin and thrombin. *J Biol. Chem.* 1966. *241* 5102–5107

SHARP A.A. B.A. WARREN A.M. PAXTON and M.J ALLINGTON Anti-coagulant therapy with a purified fraction of Malayan pit viper venom. *Lancet.* 1968 *1* 493–499

SHEIDEGGER, J.J., Une micro-méthode de l'immuno-électrophorèse *Int. Arch. Allergy* 1955 7 103–110

SHULMAN N R., A proteolytic inhibitor with anticoagulant activity separated from human urine and plasma. *J Biol. Chem.* 1954 *213* 655–671

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 401

VASODILATOR MECHANISMS IN THE SMALL INTESTINE

An experimental study in the cat

BY

BJÖRN BIBER

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 401

From the Department of Physiology
University of Göteborg
Göteborg, Sweden

VASODILATOR MECHANISMS IN THE SMALL INTESTINE

An experimental study in the cat

BY

BJÖRN BIBER

Göteborg 1973

This summary is based on studies reported in the following papers:

I Studies on the intestinal vasodilatation observed after mechanical stimulation of the mucosa of the gut
B Biber O Lundgren and J Svanvik Acta physiol scand 1971 82 177-190

II Intestinal vasodilatation in response to transmural electrical field stimulation
B Biber J Fara and O Lundgren Acta physiol scand 1973 87 277-282

III Intestinal vascular responses to 5 hydroxytryptamine
B Biber J Fara and O Lundgren Acta physiol scand 1973 87 526 534

IV Vascular reactions in the small intestine during vasodilatation
B Biber J Fara and O Lundgren Acta physiol scand 1973 In press

V A pharmacological study of intestinal vasodilatory mechanisms in the cat
B Biber J Fara and O Lundgren Accepted for publication in Acta physiol scand

VI The effects of intestinal vasodilator mechanisms on the rate of ^{85}Kr absorption in the cat
B Biber Accepted for publication in Acta physiol scand

The papers are referred to in the text by their Roman numerals

[illegible]

CONTENTS

INTRODUCTION 5

METHODOLOGICAL CONSIDERATIONS 8

LOCAL NERVOUS CONTROL OF THE INTESTINAL VASCULATURE 10

Results and comments 10
Evidence for a local nervous vasodilator mechanism in the small intestine 10
Effects on consecutive vascular sections 12
Effects on intestinal absorption and on parallel-coupled vascular sections of the gut 12

HORMONAL CONTROL OF THE INTESTINAL VASCULATURE 14

Results and comments 14
Effects on series-coupled vascular sections 14
Effects on intestinal absorption and on parallel-coupled vascular sections 15
Comparative aspects on the different vasodilator responses in the intestine 16

PHARMACOLOGICAL PROPERTIES OF THE INTESTINAL VASODILATATION 18

Results and comments 18
Pharmacological analysis 18
The possible role of 5-HT for the intestinal hyperemia 19

SUMMARY AND CONCLUSIONS 24

ACKNOWLEDGEMENTS 25

REFERENCES 26

INTRODUCTION

Vascular smooth muscle tone and hence also resistance to blood flow is the net result of several different influences The basal vascular tone is considered to be determined primarily by the myogenic activity within the vascular smooth muscle cells (Folkow 1964) the degree of which differs between various vascular sections Changes in transmural pressure caused by e g a change of arterial blood pressure alters the smooth muscle tone by modifying this myogenic activity An increased distension for example provides a positive feed back stimulus tending to enhance vasoconstriction

The remote control mainly exercised by the sympathetic nervous system adjusts the circulation to the requirements of the body as a whole largely regulating total vascular resistance often in competition with local control mechanisms Sympathetic vasoconstrictor fibres also influence the tone of venous capacitance vessels that provides a dynamic control of the proper filling of the cardiac pump Further more hormonal factors constitute another type of remote control system which in some vascular beds is of importance for the regulation of vascular smooth muscle tone

At the local level the interplay between the mechanically re inforced myogenic activity and regionally produced chemical vasodilator factors ascertains a blood flow that is closely adjusted to the current demands for oxygen and nutritients in the tissues Most of our current knowledge about the local chemical control of vascular smooth muscles is based on experiments performed on the skeletal muscle and these studies suggest that certain key substances such as CO_2 adenosine compounds potassium and hydrogen ions are of importance (Hilton 1962 Folkow and Neil 1971) An increased tissue concentration of these agents causes a relaxation of vascular smooth muscle Tissue hyperosmolarity has recently been proposed to be of particular importance in explaining exercise hyperemia (Lundvall 1972)

Not seldom textbook coverage of the local control of circulation implies that the studies of the skeletal muscular circulation may directly apply to all systemic vascular circuits However several investigations during the last decades suggest that the balance between different mechanisms may vary considerably in different organs Thus hydrogen ions have been inferred as the principle chemical vasodilator influence in the brain (Fencl et al 1968 Ingvar et al 1968) while adenosine compounds have been suggested to be of particular importance for the control of the coronary vascular bed (Berne 1964) In some glands like the salivary glands the production of specific polypeptide vasodilators kallidin and bradykinin (Hilton and Lewis 1956 Gautvik 1970) are thought to exert a dominant influence

As regards the intestinal circulation it has been repeatedly demonstrated that intake of food results in a moderate functional hyperemia increasing total splanchnic blood flow 50-300 per cent (Brodie et al 1910 Brodie and Vogt 1910 Herrick et al 1934 Lowenthal et al 1952 Brandt et al 1955 Bensadoun and Reid 1962 Fronek and Stahlgren 1968 Burns and Schenk 1969, Lunderquist Lunderquist and Nommensen 1969) This splanchnic blood flow increase usually takes place at an unchanged or only slightly increased cardiac output heart rate and blood pressure (Burns and Schenk 1969 Fronek and Stahlgren 1968 Vatner et al 1970) Few studies have however been devoted to the underlying causes of this local increase of gut blood flow It should be stressed in this context that blood circulation to the gut does not only deliver oxygen and other nutrients to the gastrointestinal tissues but it also constitutes the major transport route for absorbed substances and delivers all the material needed for secrete formation

It is of considerable interest that absorption the major function of the gut is carried out mainly by the epithelial cells of the villi which are situated relatively far away from the mucosal-submucosal arterioles the main determinants of intestinal flow resistance Hence it seems less probable that the functional hyperemia of the gut should be induced exclusively by the various agents discussed above which are essentially related to the level of metabolism Recent experimental work in this and other laboratories has also suggested that more specific mechanisms are at least in part responsible of the vasodilatation seen in the small intestine during digestion and one aim of the present series of experiments was to elucidate this particular question

Thus in paper I experimental support was presented for the existence of a local nervous vasodilator reflex in the intestinal wall which may be activated by even light mechanical stimulation of the intestinal mucosa Via this reflex intestinal blood flow may for example be locally increased by the presence of food in the gut lumen The same local reflex arch could apparently be activated directly by transmural electrical field stimulation (II) A pharmacological analysis of this reflex vasodilatation (I II V) suggested that it was possible to abolish the vascular response by blocking the intestinal receptors to 5-HT (5-hydroxytryptamine serotonin)

There are recent reports suggesting that other mechanisms than the abovementioned nervous reflex may be of particular importance in explaining the functional hyperemia of the gut Fara and coworkers (Fara et al 1972) provided experimental support for the view that also the hormones cholecystokinin (CCK) and secretin released as a result of presence of food in the intestine increases intestinal blood flow In some of the present studies a comparison was made between the

mentioned local neurogenic and these hormonally induced vasodilatations (IV V VI) The pharmacological analysis revealed that also the intestinal vasodilatations caused by the mentioned hormones could be abolished by a 5-HT blockade This suggests that 5-HT may be of common importance for the intestinal functional hyperemia and a particular study was therefore devoted to the effects of 5 HT on the intestinal vasculature as studied both during in vitro and in vivo conditions (III)

The intestinal vascular bed may be considered as a set of parallel coupled circuits supplying the different layers of the intestinal wall Each of these intramural vascular circuits consists in turn of specialized sections coupled in series such as resistance exchange and capacitance vessels (Folkow 1967) The circulatory adjustments caused by the local nervous reflex and the abovementioned hormones were analysed in paper IV with regard to their influence on the series coupled vascular sections utilizing a plethysmographic technique An indirect approach to the study of the intramural parallel-coupled vascular circuits was utilized in paper VI in which mucosal blood flow was evaluated by measuring the rate of absorption from the gut lumen of an inert lipidsoluble radioactive substance ^{85}Kr a technique that has earlier been analysed in more detail with respect to direct blood flow measurements (Svanvik 1973)

Preliminary reports of this series of investigations have been published previously (Biber et al 1970 Biber et al 1972)

METHODOLOGICAL CONSIDERATIONS

The experiments were performed on cats of both sexes deprived of food for at least 24 hours and without any obvious signs of intestinal disease Anesthesia was induced with ether and maintained with i v chloralose (50 mg/kg b w) The experimental procedures have been described fully in papers I-VI and only a brief review of the methods employed is presented below

A Operative procedures After tracheotomy a tracheal cannula was inserted and the abdomen was opened through a midline incision A jejunal segment close to the flexura duodeno jejunalis and weighing 15-30 g was chosen for the experiments The segment was left in situ with intact vascular supply while the rest of the intestine the spleen and the greater omentum were removed Venous outflow from the jejunal segment and its lymph nodes was measured by an optical drop recorder unit the blood being returned via a funnel connected to the external jugular vein Arterial blood pressure was monitored from one femoral artery by means of a mercury manometer or a Statham P23 AC pressure transducer I a injections or infusions to the jejunal segment could be made through a cannula placed in a small branch of the superior mesenteric artery In most experiments the influence of the autonomic intestinal innervation was eliminated by sectioning the nerves surrounding the superior mesenteric artery The adrenal glands were denervated or entirely excluded from the circulation by ligatures

Intestinal intraluminal pressure changes could be measured through an intraluminal catheter connected to a Statham low pressure transducer (P23 BC) both ends of the intestinal segments being then tied off

B Mechanical mucosal stimulation and transmural electrical field stimulation To achieve a mechanical stimulation of the intestinal mucosa a piece of soft plastic tube with blunt ends (length 3 cm; outer diameter 5 mm) was used in most experiments The plastic tube could be cautiously moved along the jejunal lumen by means of soft strings attached to its ends (I)

Transmural electrical field stimulation (II) was made possible through two specially designed silver electrodes The inner one was coiled along the full length of a small plastic tube and placed in the lumen to be used as a cathode The outer one was formed by numerous semicircular pieces of flattened silver wire and so placed that it closely enwrapped the intestine The intestinal segment and the electrodes were placed in a lucite chamber and covered by bodywarm Tyrode solution Stimulation was delivered through a specially constructed constant current generator operated from a Grass stimulator (model S5)

C Plethysmographic technique Plethysmographic studies (III IV) of the intestinal vasculature were performed using the method described by Folkow et al (1963) and Haglund and Lundgren (1972) A jejunal preparation was placed in situ into a lucite plethysmograph filled with bodywarm Tyrode solution making it possible to follow continuously changes in tissue volume Smaller technical modifications described in papers III and IV enabled measurement of changes in tissue volume and capillary filtration coefficient (CFC) also during transmural electrical field stimulation and mechanical mucosal stimulation

D Absorption of ^{85}Kr The intestinal uptake of an inert and rapidly diffusible radioactive tracer ^{85}Kr from the intestinal lumen was estimated during intestinal vasodilatation (VI) using a technique described by Biber et al (1973 b) Saline containing ^{85}Kr was perfused at a high rate through the jejunal lumen and the tracer amount absorbed was estimated from its appearance in the venous outflow from the jejunum and monitored continuously by means of a well type scintillation counter As the isotope was almost completely eliminated from the blood after a single pulmonary passage the arterial tracer concentration was negligible so that the ^{85}Kr absorption rate could be calculated from the venous outflow and its tracer concentration

LOCAL NERVOUS CONTROL OF THE INTESTINAL VASCULATURE

In experiments primarily performed for other purposes it was noticed that the introduction of a slender plastic tubing into the gut lumen induced a marked increase of intestinal venous outflow (Biber et al 1970) This observation represented the starting point of the studies reported in papers I and II The involvement of the central nervous system in this type of vasodilatation could be excluded first because these experiments were performed on acutely denervated intestinal segments second because no exogenous cholinergic or adrenergic vaso dilator fibres have been demonstrated in the cat small intestine (Kewenter 1965) Hence this type of intestinal vasodilatation was likely to be brought about by strictly local mechanisms

Results and comments

A Evidence for a local nervous vasodilator mechanism in the small intestine It was demonstrated in paper I that even light mechanical stimulation of the mucosa of a denervated jejunal segment caused an increase in blood flow amounting to 30-140 per cent of control Direct visual observation of intestinal segments cut open indicated that the increased blood flow certainly involved the mucosa though also other layers may have been involved The vasodilatation exhibited a rapid onset and could last for up to 15 min although the mechanical stimulations never lasted more than 30 sec

A pharmacological analysis reported in paper I strongly suggested that intramural nervous structures were involved in the hyper emia induced by mechanical stimulation of the intestinal mucosa since tetrodotoxin that blocks nerve conductivity abolished completely the vasodilator response to mechanical stimulation Moreover a similar blockade was accomplished by intraluminal administration of lidocaine another agent blocking nerve conductivity As regards the transmitter substance in the proposed nervous pathway it was suggested that neither cholinergic nor adrenergic receptors or synapses were involved It was on the other hand possible to abolish the intestinal hyperemia by bromo-LSD a 5 HT receptor blocking agent This finding will be discussed in some detail in a following chapter

If the mentioned intestinal vasodilatation is mediated by an intra mural nervous mechanism it should be possible to excite the nerve directly by appropriate electrical stimuli As reported in paper II an intestinal hyperemia could be elicited by transmural electrical field stimulation with an inner cathode electrode placed in the lumen and an outer one enwrapping the intestine which was placed in Tyrode

solution Stimulation by this procedure elicited a prompt blood flow augmentation with much the same appearance as that induced by mechanical mucosal stimulation and it exhibited identical pharmacological properties It might be argued that the application of the electrical field could have directly caused a vascular relaxation without necessitating any nervous mediation If so however the administration of tetrodotoxin or lidocaine would not affect the intestinal vasodilatation induced by electrical field stimulation but these drugs entirely eliminated this response

Several observations suggest that the mechanical mucosal stimulation and electrical field stimulation evoke vasodilatations via strictly local nervous mechanisms First acute denervation of the jejunal segment under study did not abolish the vasodilator responses Second it was shown in paper II that the completely isolated crossperfused intestine still responded with a blood flow augmentation upon electrical field stimulation

As regards the anatomical arrangement of the nervous structures involved either one of two possibilities a priori seems probable The arch may be arranged in a manner resembling the peristaltic reflex (Kottegoda 1969) i e an intramural reflex arch with one or several synapses An axon reflex similar to that described for the skin (e g Folkow Ström and Uvnäs 1950) seems to be the other possible arrangement In order to differentiate between these two possibilities experiments were performed in which cats were operated under sterile conditions all nerves along the superior mesenteric artery being sectioned accomplishing a total postsynaptic adrenergic and presynaptic cholinergic denervation The animals were allowed to recover and two weeks later when the cut distal parts of the axons had degenerated the animals were again used for investigations as described in paper I The completeness of the nerve degeneration was ascertained by the total abolishment of intestinal vascular responses to splanchnic nerve stimulation The vasodilator response to mechanical mucosal stimulation was however unaffected suggesting that structures of the axon reflex type were not involved

No consistent motility changes as measured from intraluminal pressure or tissue volume alterations were recorded in response to electrical field stimulation Spontaneous motility was transiently inhibited at the beginning of the stimulation

To summarize it is concluded from these experiments that transmural electrical field stimulation evokes a marked intestinal vasodilatation via an intramural nervous reflex arch which during physiological conditions probably is evoked by mechanical mucosal stimulation

In this connection it should be recapitulated that Ohkawa and

Prosser (1972) recently demonstrated bursts of increased nerve discharge in the submucous and myenteric plea of the cat small intestine in response to luminal distension Such an increased discharge was also elicited by means of direct mechanical stimulation of the submucosa and myenteric plexus layers Ohkawa and Prosser further demonstrated that the increased nerve discharge was accompanied by hyperpolarisation and inhibition of the circular smooth muscles Such inhibitory neurons to the intestinal smooth muscle layers, also being non-adrenergic and non-cholinergic can apparently also be stimulated directly through an electrical field (Bülbring and Tomita 1967 Rikimura and Suzuki 1971) the response being blocked by tetrodotoxin and the local anesthetic agent cocaine

B Effects on consecutive vascular sections The reactions of the intestinal consecutive vascular sections (resistance exchange and capacitance vessels) during vasodilatation evoked by mechanical mucosal and electrical field stimulation were studied by means of the plethysmographic technique (IV)

Both experimental procedures induced a rapid and large dilatation of the intestinal resistance vessels (see above) Concomitantly there was a dilatation of the capacitance vessels during field stimulation corresponding to a 10–15 per cent increase of the regional blood volume (cf Folkow et al 1963) It was not possible to estimate the blood volume changes of mechanical stimulation since the stimulation procedure itself induced large alterations of tissue volume No attempt was made to differentiate between passive and active factors as regards the recorded dilatation of the capacitance vessels (cf Öberg 1967)

The two different vasodilator procedures caused a considerable increase of the capillary filtration coefficient (CFC) of the intestine The magnitude of CFC is a function of the number and size of pores per capillary surface area but also of the number of capillaries open for perfusion This perfused area is determined by the tone of the so called precapillary sphincter sections

The increased CFC during the abovementioned vasodilatory procedures could thus be brought about by changes in either capillary permeability or in precapillary vascular tone or by a combination of both No attempts were made to differentiate between these possibilities though no clearcut indications of any increased capillary permeability were seen

C Effects on intestinal absorption and on parallel coupled vascular sections of the gut A close relationship between the magnitudes of

intestinal blood flow and intestinal absorption has been demonstrated particularly for lipid soluble substances In this respect, the mucosal exchange vessels have a key function and circulatory adjustments of this vascular section are usually well reflected in the absorption rate (cf Svanvik 1973)

In this investigation the uptake of a radioactive lipid-soluble inert tracer ^{85}Kr was recorded during intestinal vasodilatation induced by electrical field stimulation (VI) The absorption of ^{85}Kr was found to increase in proportion to the blood flow The observed increase of absorption rate was, however considerably higher than that recorded during a corresponding increase of total intestinal blood flow induced by isopropylnoradrenaline infusion (Biber et al 1973b) Furthermore during the vasodilatation caused by field stimulation a larger fraction of intestinal blood flow became fully equilibrated with the luminal contents compared with control During isopropylnoradrenaline induced vasodilatation, on the other hand a decreased equilibrated fraction was observed (Biber et al 1973b)

These two observations indicate that electrical field stimulation preferentially increases blood flow in the absorptive layers of the intestinal mucosa This in turn suggests that the studied vasodilatation may be physiologically important during digestion

HORMONAL CONTROL OF THE INTESTINAL VASCULATURE

It has been suggested since long that intestinal blood flow is influenced by hormones from the intestinal tract (Grossman 1950, Holton and Jones 1960) but this has been demonstrated convincingly first after that the involved hormones became available in a chemically pure form Thus intravascular administration of secretin and cholecystokinin (CCK) produce intestinal vasodilatation (Fara et al 1969 Ross 1970 Chou et al 1972b Fasth et al 1973) Furthermore an increased mesenteric blood flow has been reported in response to i v glucagon though the amounts necessary to produce this vasodilatation appear to greatly exceed the physiological dose range for this hormone (Tibblin et al 1970)

The significance of hormonal effects in explaining the postprandial hyperemia of the gut was investigated by Fara and coworkers (1972) These authors provided experimental support for the view that the functional mesenteric hyperemia is at least in part secondary to the release of CCK and secretin This was for example suggested by the observation that intraluminal perfusion of solutions containing L-phenylalanine or hydrochloric acid induced an intestinal hyperemia which in all probability was secondary to a physiological release of secretin and CCK (Fara et al 1972)

Since several mechanisms maybe in cooperation can be of importance for the development of the intestinal functional hyperemia it seemed to be of interest to compare the vascular effects caused by the proposed local nervous reflex with that induced by the mentioned hormones Such a comparison was hence performed as regards the reactions of the consecutive vascular sections in paper IV and as regards the rate of ^{85}Kr absorption in VI These results are summarized and discussed below while the pharmacological analysis of the vasodilator mechanisms are reported in the next chapter

Results and comments

A Effects on series-coupled vascular sections The intraarterial or intravenous infusions of physiological amounts of CCK or secretin (4-12 u/kg x min) induced a clearcut dilatation of the intestinal resistance vessels (IV) A vasodilatation of a similar magnitude was recorded when a physiological release of CCK and secretin was induced by intraluminal perfusion of L-phenylalanine and hydrochloric acid performed in the same way as by Fara and coworkers (see above) Concomitantly to this dilatation of the resistance vessels a dilatation of the capacitance vessels (mainly veins) occurred being particularly

evident during the intraarterial administration of the hormones which offers a more distinct rise in hormone concentration It was not further analyzed to what extent this increase of regional blood volume was active as a result of hormonally induced relaxation of the vascular smooth muscles or due to a passive venous distension secondary to the downstream increase of transmural pressure The effects on the capacitance vessels were quantitatively the same as those observed in connection with the vasodilatations described in the previous chapter

A considerable increase of the capillary filtration coefficient (CFC) was also demonstrated in response to i a infusions of CCK and secretin For any given blood flow level the increased CFC was always higher than control and sometimes it was apparent before the blood flow increase At lower infusion rates a CFC increase could be noticed even in the absence of any significant blood flow increase Corresponding results were obtained during intraluminal perfusion of HCl and L-phenylalanine

As discussed in paper IV and in the previous chapter the increase in CFC may partly reflect a dilatation of precapillary sphincters partly an increased capillary porosity No attempts were made to differentiate between these two possibilities However an increased porosity was suggested by the abovementioned observation that at low i a doses of CCK and secretin CFC increased even in the absence of a flow augmentation This conclusion may also be substantiated by the slow continuous increase in tissue volume seen during the intra vascular infusion of CCK and secretin However other mechanisms may explain this observation e g an increased mean capillary hydrostatic pressure secondary to precapillary vasodilatation (for a detailed discussion see paper IV)

To summarize CCK and secretin exogenously administered or endogenously released dilate resistance vessels precapillary sphincters and capacitance vessels in the intestinal vascular bed The two hormones may also increase capillary permeability by augmenting the size of the capillary pores

B Effects on intestinal absorption and on parallel-coupled vascular sections The effect of CCK and secretin on the rate of ^{85}Kr absorption was investigated in the same way as described in the preceeding chapter for the local nervous reflex The results recorded during the hormonally induced hyperemia was similar to that of the nervously produced i e an augmented rate of ^{85}Kr-absorption was observed during the vasodilatation This augmentation was greater than that seen when the same increase in total intestinal blood flow was induced by isopropylnoradrenaline suggesting that the hormones preferentially increased blood

flow in the absorptive parts of the mucosa

C Comparative aspects on the different vasodilator responses in the intestine From the results reported in this and the previous chapter it seems obvious that the vasodilatation induced reflexly or by CCK and secretin involve a pattern of similar reactions in the different series-coupled vascular sections of the intestine Moreover all the vasodilator responses studied induced an increased rate of ^{85}Kr absorption which was greater than that seen during vasodilatations evoked by isopropyl noradrenaline This resemblance in between them is less likely to be incidental but rather suggests that these particular vascular reactions substantially contributed to the intestinal hemodynamic adjustments occurring in the post prandial situation and that different mechanisms are jointly at hand to fully ascertain the intestinal functional hyperemia Depending on the nature of the intraluminal contents during digestion either chemical or mechanical stimuli may dominate It further seems probable that the release of CCK and secretin which is induced by the presence of food in the intestine causes an increase of blood flow throughout the intestine The local nervous reflex on the other hand induces an intestinal hyperemia that is largely confined to the site of the food bolus

In a preliminary study a recently developed indicator-dilution technique for measuring intestinal mucosal blood flow and mean transit time (Biber et al 1973a) has been used during vasodilatations induced by CCK and secretin or by transmural electrical field stimulation The method involves intraarterial injections of an intravascular β-radiating tracer and radioactivity measurements by means of a cylindrical detector placed inside the intestinal lumen The results so far suggest that the mean transit time in the villous part of the mucosa is significantly decreased during the mentioned types of intestinal vasodilatations and is even lower than that obtained during isopropylnoradrenaline hyperemia of corresponding magnitude From previous studies (Svanvik 1973) it has become evident that the absorption rate of ^{85}Kr is well correlated to the mean transit time and hence to the linear blood flow rate in the mucosa while regional blood volume and diffusion are of relatively less importance This was suggested to reflect the influence of a countercurrent diffusion exchange which takes place between ascending and descending vessels in the villi and adjacent parts of the mucosa and which delays net intestinal absorption (Lundgren 1967) According to this hypothesis the time available for exchange diffusion is determined by the linear flow rate in the villi and the hindrance to absorption offered by this exchange diffusion would hence decrease at higher linear flow rates The increased absorption in

paper VI could thus partly be caused by a reduced efficiency of the villous countercurrent exchanger Further investigations are in progress to elucidate this matter

PHARMACOLOGICAL PROPERTIES OF THE INTESTINAL VASODILATATION

In the preceding chapters two main types of vasodilatation have been described one apparently induced via a local nervous reflex arch one involving hormonal mechanisms It is believed that both these mechanisms play a physiological role in the functional hyperemia of the gut This chapter deals mainly with possible mediator links in the two types of hyperemia To elucidate this question a pharmacological analysis of the intestinal vasodilatation have been performed particularly with respect to the significance of 5-HT

Results and comments

A Pharmacological analysis The local vasodilator nervous arch which was activated by slight mechanical stimulation of the mucosa or by transmural electrical field stimulation involved no adrenergic or cholinergic receptor sites either at the effector cells or in the ganglionic synapses (I II) The same was true for the intestinal blood flow increase induced by CCK or secretin It was possible however to abolish the vasodilator response to mechanical mucosal stimulation by administration of LSD a 5 HT blocking drug (I) This drug blocked also the vasodilatation induced by transmural electrical field stimulation as was evident from the 4 experiments in which it could be used Due to the inavailibility of 2 bromo LSD 5 HT blockade had to be accomplished by dihydroergotamine (DHE) in the remaining part of this investigation (V) However also DHE completely abolished the intestinal vascular response to local administration of exogenous 5 HT (III) which was utilized as a test of the successfullness of the blockade (Gyermek 1969) DHE have also adrenergic α-receptors blocking properties but first in higher doses than those necessary to block 5 HT receptors

As reported in paper V the intestinal vasodilator response to transmural field stimulation and to administration of exogenous CCK or secretin was totally abolished by 5-HT blockade utilizing 2 bromo LSD and DHE However the only 5 HT blocker considered to be fully specific is 5-HT itself Therefore to obtain further evidence concerning the 5-HT dependence of the vasodilator responses studied a series of experiments were performed with induced tachyphylaxis of the intestinal vascular effects to exogenous 5 HT (V) From these experiments it became evident that a preserved vascular response to 5-HT in the intestine is a prerequisite for the development of the different vasodilatations of this study Therefore a common physiological role of 5 HT in the development of the hormonally and nervously induced vasodilatations was suggested

Supporting this hypothesis it was found (paper III) that exogenous

5 HT either *given as an intraarterial injection (10 µg) to the intestine* or as a slow i a infusion (10-25 µg/min) produced the same pattern of intestinal vasodilatation concerning the consecutive vascular sections as did CCK, secretin mechanical stimulation of the mucosa and electrical field stimulation (IV) Like these latter vasodilator responses the 5-HT vasodilatation was unaffected by cholinergic and adrenergic pharmacological receptor blockade. However the 5-HT vasodilator response is *probably nerve-mediated as it was completely but reversibly* inhibited by local nervous blockade with tetrodotoxin (III) or lidocaine (unpublished observations) Actually in some of these experiments an intestinal vasoconstriction was observed in response to 5 HT during nervous blockade

The results reported above may give the impression that all the different types of vasodilatation are elicited via a common nervous mechanism dependent on an intact 5-HT receptor function To test this hypothesis experiments were performed in which the effects of tetrodotoxin on the hormonally induced vasodilatations was studied As reported in paper V tetrodotoxin did not interfere with the hormonal effects Thus the existence of a vasodilator mechanism common to all types of intestinal hyperemia studied seems less likely Instead it appears as if two separate mechanisms causing vascular relaxation in the intestine have to be distinguished although the ultimate vascular response exhibits several qualities in common

B The possible role of 5-HT for the intestinal hyperemia Numerous reports concerning 5-HT and its physiological role have been published since the synthesis of the substance was accomplished in 1951 by Hamlin and Fisher (see e g reviews by Erspamer 1966 Page 1968 Penttilä 1966 Thompson 1971) In the normal small intestine 5-HT *appears to be stored mainly within the intestinal enterochromaffin cells* which are scattered among the epithelial cells lining the crypts of Lieberkühn and those covering the villi (cf Fig 1) However it also seems probable that 5-HT is normally localized in the myenteric plexus but in quite small amounts usually not detectable by ordinary histochemical methods (Tafuri and Raick 1964 Gershon and Ross 1966 Hammarström et al 1966 Gershon 1970 Gershon and Altman 1971 Robinson and Gershon 1971)

An unanimous agreement on the functional importance of intestinal 5-HT is yet not at hand 5-HT has been proposed to participate in the regulation of intestinal motility either as a transmitter released at the smooth muscle cells or in synapses (Kottegoda 1969 Bülbring 1970) The effect of 5-HT on blood vessels are somewhat variable The in vivo reactions on the vasculature of several organs usually involves

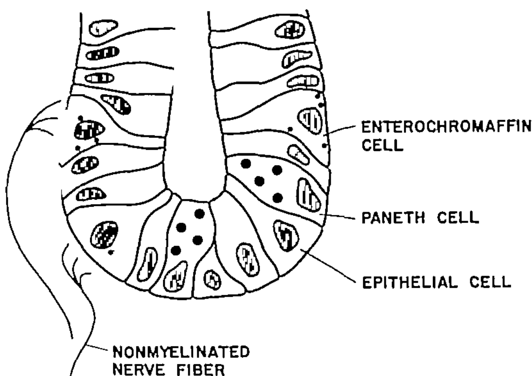


Fig 1 Epithelium at the base of a crypt in the intestinal mucosa with enterochromaffin cells

a vasoconstriction particularly with respect to larger vessels This was also found to be the case when cat mesenteric arcuate arteries and veins were studied in vitro (III) while 5-HT in vivo caused an intestinal vasodilatation In which situation the responses of the small resistance and capacitance vessels dominate

Concerning the vasodilator mechanisms caused by mechanical mucosal stimulation or by transmural field stimulation, a functional model was proposed in paper V as a basis for further study In short mechanical stimulation of the mucosa which is likely to occur during digestion excites an intramural neuronal arch (Fig 2) which involves 5 HT as a synaptic transmitter and possibly also as a stimulus for mucosal nerve endings as a result of 5 HT release from enterochromaffin cells in the mucosa The electrical transmural field stimulates such

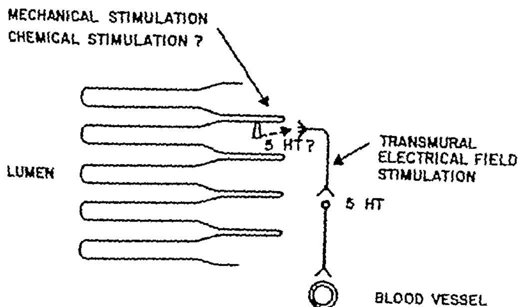


Fig 2 Hypothetical arrangement of a nervous vasodilator reflex arch in the intestine The number of neurons in each arch is arbituarily set to two For details see text!

---

structures directly evidently before the synaptic 5-HT junction (see Fig 2 Considerable circumstantial evidence for such a model can be presented:

1 Exogenous 5-HT apparently causes a nerve-mediated intestinal vasodilator response which as far as it was possible to investigate on parallel coupled and consecutive vascular sections was similar to that induced by mechanical mucosal or electrical field stimulation (III IV)

2 The vascular response to mechanical mucosal and electrical field stimulation being also nerve-mediated was reversibly inhibited by pharmacological 5-HT blockade or by vascular 5-HT tachyphylaxis

3 5-HT occurs naturally in the enterochromaffin cells of the intestinal mu cosa where enzymes for its production are available Fig 1 shows schematically the anatomy of a crypt of Lieberkühn where the entero-chromaffin cells with their basal 5-HT containing granulae are positioned in between Paneth cells and ordinary epithelial cells Small nonmyelinated nerve fibres are located in close contact with the basal membrane of the enterochromaffin cells as was first suggested by Masson (1928) from light microscopy studies and later confirmed by means of electron microscopy techniques (e g Gasbarrini et al 1969) Some of the small nerve fibres in the mucosa are obviously of intra mural origin arising from the myenteric and submucous plexa (Scho field 1960)

4 Small amounts of 5-HT are also present and actively taken up in the myenteric plexus the precise localization being probably intra neuronal Additionally Baumgarten et al (1970) have by means of electron microscopy demonstrated non-cholinergic and non-adrenergic neurons called p-fibres (polypeptide fibres) in the myenteric plexus The axon varicosities of these neurons which establish numerous syn-aptic contacts are suggested to contain a polypeptide substance (cholecystokinin? secretin?) and 5-HT (Baumgarten et al 1970 Robinson and Gershon 1971)

5 Mechanical mucosal stimulation causes liberation of 5 HT from the intestine into the vasculature (Burks and Long 1966) as does transmural electrical field stimulation in vivo (V) Moreover feeding, probably involving mechanical mucosal stimulation of the intestine leads to an increased 5-HT concentration in the portal blood (Smith 1958 Black et al 1959) Fasting on the other hand minimizing mucosal stimu lation, augments the 5-HT content in the intestinal mucosa (Thompson and Campbell 1967) and intestinal wall in general (Gal and Drewes 1961 Lemmer and May 1971)

6 An increased neuronal discharge that inhibits at least intestinal visceral smooth muscle has been demonstrated in response to intestinal distension or other types of mechanical stimulation within the in testinal wall (Ohkawa and Prosser 1972)

The number of neurons in each unit of the proposed nervous arch is unknown as is the nature of the non-cholinergic and non adrenergic transmitter at the vascular receptor site It should how ever in this connection be pointed out that adenosine triphosphate or a related nucleotide has been proposed to be the transmitter sub-stance of the nervously mediated inhibition of gastric smooth muscle (receptive relaxation Burnstock 1972) and the participation of such a sub-

stance as a transmitter also at the vascular receptor site of the suggested reflex arch in the intestine is not impossible

The possibility that also intraluminal chemical factors can activate the proposed reflex arch is indicated in Fig 1 Results in agreement with this suggestion was reported by Chou et al (1972 b) who demonstrated that the intraluminal installation of hypertonic glucose causes a nervemediated vasodilatation in the small intestine of the dog Furthermore in paper I, it was reported that bile slowly introduced into the gut lumen caused a prolonged intestinal vasodilatation

As regards the hormonally induced vasodilatation, the present results clearly indicate that the intestinal vascular actions of CCK and secretin require an intact reactivity to 5-HT However in contrast to 5-HT itself the two hormones induce vasodilatation independent of *intramural nervous structures It is difficult to reconcile these observations* but they may be explained by a hypothesis that released CCK and secretin relax vascular smooth muscle in the intestine only when acting together with 5-HT It has been suggested that enterochromaffin cells and p-fibres in the myenteric plexus contain both 5-HT and poly peptide hormones and a simultaneous release of these substances may occur (Baumgarten et al 1970 Robinson and Gershon 1971 Thompson 1971) Also a reduced content of 5-HT in the intestine has been reported after an intraluminal HCl administration (Resnick and Gray 1962) which has been demonstrated to release also CCK and secretin (Fara et al 1972) The significance of these latter findings as well as the true physiological relationship between intestinal hormones and 5-HT has however yet to be elucidated

SUMMARY AND CONCLUSIONS

The present series of experiments was carried out to explore possible mechanisms underlying the post prandial hyperemia of the gut and to investigate the hemodynamic reactions induced by these mechanisms The results can be summarized as follows

1 Light mechanical stimulation of the mucosa was demonstrated to cause a localized intestinal vasodilatation obviously through the activation of a local intramural non-cholinergic and non-adrenergic nervous reflex arch (Fig 2) This nervous structure which could be stimulated directly by applying an electrical field across the intestinal wall was suggested to participate in the development of post prandial hyperemia

2 5 HT (5-hydroxytryptamine; serotonin) which is abundantly present in the enterochromaffin cells of the intestinal mucosa and probably to some extent also in the myenteric plexus was shown to be a potent vasodilator of the intestinal vasculature Judging from a pharmacological analysis 5 HT is directly involved in the reflexly elicited vasodilatation as a neurotransmitter and/or as a stimulus at nerve endings in the mucosa (Fig 2)

3 The intestinal polypeptide hormones cholecystokinin (CCK) and secretin are known to cause vasodilatation throughout the intestine when released in response to intraluminal chemical stimuli In the present study it was demonstrated that an intact vascular response to 5-HT is necessary for the vasodilatory effects of CCK and secretin

4 Pletysmographic investigations showed that the reactions within the consecutive vascular sections (resistance exchange and capacitance vessels) in the intestine were similar whether a vasodilatation was induced by CCK and secretin or by activation of the intramural nervous vasodilator arch

Also the different vasodilatory responses in question caused an increased uptake of a lipidsoluble inert radioactive tracer ^{85}Kr from the intestinal lumen The uptake of the tracer during nervously or hormonally induced vasodilatations was higher than during an intestinal hyperemia of corresponding magnitude induced by isopropylnoradrenaline It was hence suggested that local chemical and mechanical stimulation of the mucosa leads to such a redistribution of the blood flow in the gut wall as to facilitate absorption

ACKNOWLEDGEMENTS

During the course of this investigation I have received valuable help from several sources I take the opportunity to express my sincere gratitude

to Docent Ove Lundgren who introduced me into this field of research for kind guidance and inestimable support and for generously spending much of his time on my problems

to Professor Björn Folkow for giving me the benefit of his wide experience for facilitating and encouraging my work

to my coworkers Dr Joar Svanvik and guest researcher Dr [illegible] Fara for friendly assistance and many stimulating discussions

to Dr Hasse Abrahamsson who shared his room for studying and office work with me for his kind participation in our experimental investigations on man s adaptation and endurance to over-population and to many other colleagues who have allowed time for rewarding discussions

to Civ ing Lars Stage for advice and help especially in matters concerning the electrical field stimulation

to the competent technical assistants in the Department of Physiology particularly Mrs Eva Bengtsson Mrs Dagny Johansson Miss Pia Larsson and Mrs Lisbeth Lindgren

to the animal keeper Gösta Magnusson

to the photographer Tomas Palm

to Mrs Kerstin Lundgren and Mrs Sylvia Palmqvist for effective secretarial work

This research has been sponsored by grants from the Swedish Medical Research Council (B73 14X-2855-04) from the Faculty of Medicine University of Göteborg and from Svenska Sällskapet för Medicinsk Forskning

FOLKOW B Regional adjustments of intestinal blood flow Gastro-enterology 1967 52 423-432

FOLKOW B O LUNDGREN and I WALLENTIN Studies on the relationship between flow resistance, capillary filtration coefficient and regional blood volume in the small intestine of the cat Acta physiol scand 1963 57 270-283

FOLKOW B and E NEIL Circulation Oxford University Press 1971

FOLKOW B , G STRÖM and B UVNAS Do dorsal root fibres convey centrally induced vasodilator impulses? Acta physiol scand 1950 21 145-158

FRONEK, K and L H STAHLGREN Systemic and regional hemodynamic changes during food intake and digestion in nonanaesthetized dogs Circulat Res 1968 23 687-692

GASBARRINI G N MELCHIONDA F BENFENATI B.A MANTOVANI and G AURELI Studio del sistema enterocromaffine e del metabolismo triptaminico nell uomo Boll Sci med 1969 141 85-118

GAL E M and P A DREWES Studies on the metabolism of 5-hydroxytryptamine (serotonin) I Effect of starvation and thiamine deficiency Proc Soc exp Biol N Y 1961 106 295-297

GAUTVIK K Studies on vasodilator mechanisms in the submandibular salivary gland in cats Universitetsförlaget Oslo 1970

GERSHON M D The localization of 5-hydroxytryptamine and its participation in neural inhibition of gastrointestinal smooth musle Jap J Smooth Muscle Res 1970 6 113-115

GERSHON M D and R F ALTMAN An analysis of the uptake of 5-hydroxytryptamine by the myenteric plexus of the small intestine of the guinea pig J pharmacol exp Ther 1971 179 29-41

GERSHON M D and L L ROSS Location of sites of 5-hydroxytryptamine storage and metabolism by radioautography J Physiol (Lond) 1966 186 477-490

GROSSMAN M I Gastrointestinal hormones Physiol Rev 1950 30 33-90

GYERMEK L Drugs which antagonize 5-hydroxytryptamine and related indolealkylamines In: Handbook of Experimental Pharma

cology 1966 XIX 471-592

HAGLUND U and O LUNDGREN Reactions within consecutive vascular sections of the small intestine of the cat during prolonged hypotension Acta physiol scand 1972 84 151-163

HAMLIN K E and F E FISHER The synthesis of 5 hydroxytryptamine J Amer Chem Soc 1951 73 5007

HAMMARSTRÖM L M RITZÉN and S ULLBERG Combined autoradiography and fluorescence microscopy Localization of labelled 5-hydroxytryptophan in relation to endogenous 5-hydroxytryptamine in the gastrointestinal tract Experientia 1966 22 213-218

HERRICK J F H E ESSEX F C MANN and E J BALDES The effect of digestion on the blood flow in certain blood vessels of the dog Amer J Physiol 1934 108 621-628

HILTON S M Local mechanisms regulating periferal blood flow Physiol Rev 1962 42 Suppl 5 265-275

HILTON S M and G P LEWIS The relationship between glandular activity bradykinin formation and functional vasodilatation in the submandibular salivary gland J Physiol (Lond) 1956 134 471-483

HOLTON P and M JONES Some observations on changes in the blood content of the cat s pancreas during activity J Physiol (Lond) 1960 150 479-488

INGVAR D H N A LASSEN B K SIESJÖ and E SKINHÖJ Third Internat Symp on Cerebral blood flow and Cerebrospinal fluid Lund-Copenhagen Scand J Clin Lab Invest 1968 Suppl 102

KEWENTER J The vagal control of the jejunal and ileal motility and blood flow Acta physiol scand 1965 65 Suppl 251

KOTTEGODA S R An analysis of possible nervous mechanisms involved in the peristaltic reflex J Physiol (Lond) 1969 200 687-712

LEMMER B and B MAY Accumulation of serotonin by human trombocytes during starvation Horm Metab Res 1971 3 110-113

LOWENTHAL M K HARPUDER and S D BLATT Peripheral and visceral vascular effects of exercise and postprandial state in supine position J Appl physiol 1952 4 689-694

LUNDERQUIST A , A LUNDERQUIST and N NOMMESEN Angiographic changes during digestion Amer J Roentgenol 1969 107:1 191 197

LUNDGREN O Studies on blood flow distribution and countercurrent exchange in the small intestine of the cat Acta physiol scand 1967 Suppl 303

LUNDVALL J Tissue hyperosmolarity as a mediator of vasodilatation and transcapillary fluid flux in exercising skeletal muscle Acta physiol scand 1972 Suppl 379

MASSON P Carcinoids (argentaffin cell tumors) and nerve hyper plasia of the appendicular mucosa Amer J Path 1928 4 181-211

ÖBERG B The relationship between active constriction and passive recoil of the veins at various distending pressures Acta physiol scand 1967 71 233-247

OHKAWA H and C L PROSSER Electrical activity in myenteric and submucous plexuses of cat intestine Amer J Physiol 1972 222 1412-1419

PAGE I H Serotonin Year Book Medical Publishers Inc Chicago 1968

PENTTILA A Histochemical reactions of the enterochromaffin cells and the 5-Hydroxytryptamine content of the mammalian duo denum Acta physiol scand 1966 Suppl 281

RESNICK R H and S J GRAY Chemical and histologic demon- stration of hydrochloric acid induced release of serotonin from intestinal mucosa Gastroenterology 1962 42 48 55

RIKIMARU A and T SUZUKI Neural mechanism of the relaxing responses of guinea-pig taenia coli Tohoku J exp Med 1971 103 303 315

ROBINSON R G and M D GERSHON Synthesis and uptake of 5-hydroxytryptamine by the myenteric plexus of the guinea pig ileum A histochemical study J pharmacol exp Ther 1971 178 311-324

ROSS G Cardiovascular effects of secretin Amer J Physiol 1970 218 1166-1170

SCHOFIELD G C Experimental studies on the innervation of mucous membrane of the gut Brain 1960 83 490-514

SMITH A N The effect of 5 hydroxytryptamine on acid gastric secretion In 5 hydroxytryptamine Ed G P Lewis Pergamon Press Inc London 1958 183-190

SVANVIK J Mucosal blood circulation and its influence on passive absorption in the small intestine An experimental study in the cat Acta physiol scand 1973 Suppl 385

TAFURI W L and A RAICK Presence of 5 hydroxytryptamine in the intramural nervous system of guinea pig s intestine Z Naturf 1964 19 1126-1128

THOMPSON J H Serotonin and the alimenary tract Res Commun Chem Pathol Pharmacol 1971 2 687-781

THOMPSON J H and L B CAMPBELL Bowel serotonin levels in the fasted and non-fasted Sprague-Dawley rat Experientia 1967 23 67-68

TIBBLIN S N G KOCK and G SCHENK Splanchnic hemodynamic responses to glucagon Arch Surg (Chicago) 1970 100 84-89

VATNER S F D FRANKLIN and R L van CITTERS Mesenteric vasoactivity associated with eating and digestion in the con scious dog Amer J Physiol 1970 219:1 170-174

LUNDERQUIST A , A LUNDERQUIST and N NOMMESEN Angiographic changes during digestion Amer J Roentgenol 1969 107:1 191-197

LUNDGREN O Studies on blood flow distribution and countercurrent exchange in the small intestine of the cat Acta physiol scand 1967 Suppl 303

LUNDVALL J , Tissue hyperosmolarity as a mediator of vasodilatation and transcapillary fluid flux in exercising skeletal muscle Acta physiol scand 1972 Suppl 379

MASSON P Carcinoids (argentaffin cell tumors) and nerve hyperplasia of the appendicular mucosa Amer J Path 1928 4 181-211

ÖBERG B The relationship between active constriction and passive recoil of the veins at various distending pressures Acta physiol scand 1967 71 233-247

OHKAWA H and C L PROSSER Electrical activity in myenteric and submucous plexuses of cat intestine Amer J Physiol 1972 222 1412-1419

PAGE I H Serotonin Year Book Medical Publishers Inc Chicago 1968

PENTTILA A Histochemical reactions of the enterochromaffin cells and the 5-Hydroxytryptamine content of the mammalian duodenum Acta physiol scand 1966 Suppl 281

RESNICK R H and S J GRAY Chemical and histologic demonstration of hydrochloric acid induced release of serotonin from intestinal mucosa Gastroenterology 1962 42 48 55

RIKIMARU A and T SUZUKI Neural mechanism of the relaxing responses of guinea-pig taenia coli Tohoku J exp Med 1971 103 303-315

ROBINSON R G and M D GERSHON Synthesis and uptake of 5 hydroxytryptamine by the myenteric plexus of the guinea-pig ileum A histochemical study J pharmacol exp Ther 1971 178 311-324

ROSS G Cardiovascular effects of secretin Amer J Physiol 1970 218 1166-1170

SCHOFIELD G C Experimental studies on the innervation of mucous membrane of the gut Brain 1960 83 490-514

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 402

Glycogen Storage and Depletion in Human Skeletal Muscle Fibres

BY

KARIN PIEHL

STOCKHOLM 1974

Glycogen Storage and Depletion in Human Skeletal Muscle Fibres

AKADEMISK AVHANDLING

som med vederbörligt tillstånd för erhållande av filosofie doktorsexamen vid Karolinska Institutet offentligen försvaras i Fysiologiska institutionens föreläsningssal, Karolinska Institutet, måndagen den 25 februari 1974 kl. 9

AV

KARIN PIEHL
Fil. kand.

STOCKHOLM 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 402

From the Department of Physiology Gymnastik- och idrottshögskolan, Stockholm, Sweden.

Glycogen Storage and Depletion in Human Skeletal Muscle Fibres

BY

KARIN PIEHL

STOCKHOLM 1974

The present thesis is based o th following pap rs

I G ll iok P D R B Armstro g C W Saub rt IV K Piehl nd B Saltin E zyme activity d fiber oomposit in skeletal mus l of untrain d nd tr ined men J appl Physi l 1972 33 312 319

II Gollniok P D K Piehl C W Saubert IV R B Armstro g and B S ltin Diet exeroise and glyc g n changes in huma musol fibers J appl Phy iol 1972 33 421 425

III Pi hl K Time c rse for refilling of glycogen t res i huma m scle fibres followi g ex roise indu ed glyc g depleti n Acta phy i l s and 1974 00 000 000

IV Pi hl K S Adolf n and K Nazar Glyc g t r g and glycog synth tas cti ity i tr i d nd tr i d mu cle of ma A ta phy i l s d 1974 00 000 000

V G llni k P D K Pi hl nd B S lti Sel ctive glycoge d pletio patt rn i h man muscl fibre ft r exeroi e f ryi g int n ity d t aryi g p dal r tes (For p blicati 1974)

Th p p rs will b referr d t by th i R ma m r l

INTRODUCTION

The first report describing skeletal muscle composition was p blished i 1678 by Lore zi i who had notic d v ri tio i the colour of certain muscles of the rabbit (for ref see Paukul 1904) It was then supposed that the red colour noted in som m l s was du to a rich r s pply f blood Kölliker (1850) advanc d th hypothesis ubsequently confirmed by Kühne (1865) that the pigment was co tained withi the co tractile substance of the fibr s (f r ref see Busk and Huxley 1853) The ubst nce pro iding the colour was c lled myocrome by Mörner (1897) but was l t r d ignated myohemoglobin r my gl bin (Günth r 1921) Moreo er the most a tive muscl s were found to contain more of thi pigment (L kester 1871) U ing l tri l timulation of red nd white mu cles Ranvier (1873) found that red mus l contr ct d nd relax d more slowly than whit muscles Grützne (1887) a d th r in estigators st died th beh vi r f th muscle during single twitch and during t t nus Glei (1887) ugg t d differi g met b li m f r r d d white mus l In hi thesi 1891 Kn ll called the fibre prot pl sm rm nd protoplasma reich Grützner al came to the con cl sion th t the muscles of higher mammals co tained a i timat mixture of red and whit fibre nd that the r ti of th s tw fibre typ gov rn d muscle s c ntractil properti By ntr t fibre p ing the same c tr ctil propertie w re grouped in b ndles (D nny Brow 1929) Th s e rly rep rt l d to the g neral view th t red mus l s were a so iat d with s s t i d repeat d work d white mu cle with r pid igoro co tra tio of bri fer durati

In th p st three d cad s int iv hist chemic l d bi hemi l t di h ve b made on mu cle ti from everal pe i A number of muscle groups h b e mi ed nd at l st two or more fibre types h b e de rib d Ogata ha s gg ted thre diff re t fibre type b ed o th t i i g i t n ity f r oxid ti zyme i r l mammal (Og t 1958 a b c) Th ame uth r b eq ently fou d differe b twe the fibre i gly g n co t t e d gly lytic acti ity by i g

excl sively red and white muscle fibre preparations (Og ta 1960) Ev though differe ces in metabolism and substrate content of fibres were described prot in content was found to b the same This was first d termined by Meyer and Web r (1933) and was later confirmed by Bárány and his co workers (Bárány et l 1965) This latter group also found great r ATPase activity in white mu les than in red muscles a finding correlat d to a fast r spe d of contr otion by white muscles (Bárány 1967) High and low staining int nsity for myosin ATPas determined with the histochemical technique introduced by Padykula and Herman (1955) w re als fo nd to c rrelate to th peed of contraction (Barnard et al 1971) Th re ha e been a limit d number of studie of huma skel t l muscle Reports published hitherto have dealt with histo hemical fi dings nd few attempts have b en made to har oteri e fibre typ s or fibre composition in large and s ied m terial U ing histochemic l stai ing for several enzymes d s b tret s Engel (1962) described the existe ce of two fibre types type I a d typ II imil r findings were report d by Edström and Ny tröm (1969) ing the same technique Other in stigators have empl yed different histochemic l staini g m thods t id tify v ri s e zyme a d s bstrates Th omen l t e h e theref re varied as show in Table I

Glycog i th muscl w first de cribed in 1859 by Bernard Th y thes s f glycoge w st di d by Leloir d his co work r (1959) d w s f u d to be bro ght abo t thro gh an en ymati system O of the i l ed nzymes glycog n sy the t w found to b rat limiti g a d therefore a k y enzyme in glyo g y th is Glycoge s well as the tw nzymes in l ed n t reg l tio gly oge sy thetase d pho phoryl se h ve b e reported t ry b twe n fibre types i the sk l tal muscl f e er l pe i s i luding ma A brief summary is gi e i T ble II Ass m ng th t gly og sy thetase nd phosphoryl ti ty refl ct th ability of the muscle fibr t sy th ti d tiliz glycog n it seems ery lik ly th t fibre w th a high l el of thes e ymes pr domina tly u e gly g th ir rce of e e gy The white fibre h s usu lly be fo d to c t n b th more gly ogen a d more pho ph ryl (T ble II)

Table I S mmary f diff re t fibr cl sific tion y tems The ome cl t re d in the pre e t th si is b s d on th sy tem i trod ed by Barn rd et l 1971 li ted here at th t p of th t bl

Autho s	M thods	Species	Nome clatures		
B r ard et l 1971	M ltiple histochemical	guinea pig	red	intermedi te	white
Ogat 1958 b o		r bbit	int rmediate	red	white
Stein d Padyk la 1962		rat	type C	type B	type A
Romanul 1964		rat	group II	group III	group I
Clos and Sw tt 1966	Presumed c ntraction speed	cat	fast	slow	fast
Bárá y 1967	ATPase activity	se eral speci	type II	type I	type II
Burk 1967	Multiple histochemical	cat	type FR	typ S	type FF
Gauthier 1969	Electron micro c p	rat	intermediate	red	white
Peter et al 1972	Q ant enzym and substr d termi ations and multipl hist chemic l	rabbit and gui ea pig	fast twitch o idative glycolytic	slow twitch oxidative	fast twitch glycolytic
Dubowitz nd Pearse 1960	Multipl hi tochemical	everal sp i s m		red	white
Ogata and Mori 1964		e ral species man	medium	red	whit
Engel 1962	ATPa (hi tochemical)	ma		type I	type II
Jam s 1968	Myoglobin	man		high	low
Ogata nd Murata 1969	M ltiple hist chemical	man	intermediate	red	white
Edström and Nyström 1969	ATP e (hi tochemic l)	m n		typ I	type II

Table II Glycogen onte t a d gly og y th t se a d ph sphorylase a tivity i muscle fibres from varying species i cl ding ma light taining or low cti ity; medi m; dark staining or high acti ty This subj otive rating i only valid for comparisons within each tudy d a ot be d to make direct ompa is n between v rio s studies

Authors	Spe ies	Methods (hi tochemical and qu ntit ti)	Fibre types red	inter-mediate	white
				x)	
Ge rg a d N ik 1958	pige n	c rmi e; qua t gly og n			
Og ta 1960	r bbit	q a t glycog			
St in nd Padyk l 1962	rat	PAS			
K gelberg and Ed tröm 1968	rat	PAS; phosphorylase (a b)			
Gillespie Simp a d Edgerton 1970	g i a pig	q nt glycogen			
Peter et al 1972	guin a pig nd rabbit	PAS; ph sphorylase (b) qua t glycogen quant phosphorylase (a b)			
Lindholm and Pi hl 1974	hors	PAS		( )	
He s a d Pearse 1961	rat	ynthetase (I D) phosphorylase (a b)	( ) ( )		
St George Stubbs and Blanchaer 1965	guinea pig	synthetase (I D) phosphorylase (a b) quant synthetase (I D) quant phosphorylase (a b)			
Bocek nd B tty 1966	rat and monkey	synthetase (I D) phosphorylase (a b) quant synthetase (I D) q ant phosphorylase (a b)			
Dubowitz and Pearse 1960	sever l species nd man	phosphorylase (a b)	( )		
Engel 1962	man	PAS; phosphorylase (a b) synthetase (I D)			
Ed tröm and Nyström 1969	man	PAS; phosphorylase (a b)			

x) No attempt to distinguish betwe th two red fibre typ s

Conflicting reports have been published on glycog n ynthe tas Georg nd Naik (1958) reported bro d fibres to be glycog n loaded a d suggested that these fibres are adapted f r rapid and sudde action Engel (1962) reported type II fibres in human tis ue to be ri h in both glycogen and the two enzyme By c ntrast S aki and T keuchi (1963) fo d weak st ining int nsity for phosphorylas i fibres with l rg di meters Stein and Padykula (1962) sugge ted that variatio s in glycoge co tent (PAS staini g intensity) reflect v rious phas s in cycle of gly ogen st r g and release Th c sist ntly greater stai ing intensity for gly g n in type A (typ II) fibres o ld b interpreted a evidence indic ting that these fibres are the most vid ynthesiz rs or that th y are th least avid u ers of glycogen Stein and Padyk la (1962) also emph iz d th s f PAS tai ing orrelated to q antit tiv glycoge s ay in order to follow glycog depletion i mu cl fibres s s to determin in which of the fibre typ glycoge depl ti n occurred A preferential l s of glycogen was fou d i th whit or type A fibre wh n i dividual nerve fib or th tire erve or mus le of rat were subjected t l ctri l timul tio (Kugelb rg d Ed trôm 1968 Edgert t l 1970) Ex rci e in g in pig and rat was bsequ ntly f und to produce differe t glyo g n depletio patt rn in the fibres (Edgert et l 1970 b Armstrong Sh ph rd and Gollnick 1973)

On th ba is of th foreme tio d reports s mmarised i p rt i T ble I d II th fibre typ which c uld be f d in human skelet l mu cl were u rtai M re v r th gly ge t nt f the fibre st re t (torag) nd d ri g er i (depletion) i ot fully k ow Th im f th pres t st dy was th ref re to n ly a d h r t rise usi g a l rge d v ried mat ri l th fibre type a d fibre compositi i th kelet l mu cl of ma A th r aim was to ami e th u t t und r v ri d b t ntroll d onditi ns t which gly ge i st red i fibres d wh th r glyo g i grad lly d pl t d in n fibre typ r th other d ring erci

SUBJECTS

S bje ts One hundred one healthy me of different ag s (17 58 years) with mean weight f 71 (60 85) kg a d a me n height of 179 (169 193) cm were studied in these investigations They repres nted different degrees of tr ini g in regard to circula tory functi n (oxygen pt ke) encompassing completely ntrained subjects (ma imal oxyg upt ke of 32 ml (kg x min) [1] and xtremely well trained subj cts (ma imal oxyg n uptake of 80 ml (kg mi) [1] Th ir mea oxyge uptake was 57 ml (kg x min) [1] and their median oxygen ptake 52 ml (kg x min) [1]

My cles exami ed Th quadriceps femoris (astus lat ralis) and th d lt id in th determination of fibre distrib ti and the q adri ep femoris only in th examin ti n of glycogen storage a d depletion

METHODOLOGICAL CONSIDERATIONS

Pri ciple fo histo hemi al a d qu titati e d termi ati n S T ble III The meth dol gic l error of the different methods i given n p re thesis i Tabl III i c j ction with h meth d

E ercis t ts a d d t rmi tion of oxyge upt k E rci e w s perf rm d n l ctri lly brak d bicy le rgomet r O yg pt k ($\dot{V}_{O_2}$) wa me sured with th Dougl s bag proced re o ev r l ca io duri g bmaxim l x rci e le t d t repres t a gi p rc t g f h subj ct s $\dot{V}_{O_2}$ m x Ma imal o yge ptak w d termined f r e ch ubject usi g the l velli g ff rit ri (A tr d nd Salti 1961)

Bi p y t chniq Mus l mpl s were t k n si g the ne dl biop y t ch iq (B rg tröm 1962) Ski a d fa cia were se the tised with 1 1 5 ml f Xyloc in a d the i ci ion was made with a alp l blade The a th si w hardly lik ly t aff ct biochemi al a d hi t hemical says as sampl s w re lways

Table III Brief mmary of the m thods discus d in the pre nt th sis Meth d logic l error given in parenth i

A ys	M thod	Ass ys principle
Qu tit ti enzym tivity ass ys		
S ccin te d hydrogen s (EC 1 3 99 1)[x] (1 %)	Co peratein Lazarow and K rf ss 1950	Spectrophotometry; reduction of oxidized cytoctome c
Glycogen y th tase (EC 2 4 1 11) (2 %)	Villar-P lasi et l 1966 m difi d by Thomas Schlender and L rner 1968	Incorporation of UDP glucose ^{14}C into glyc ge ; ±10 mM G 6 P
H okinase (EC 2 7 1 1) (2 %)	Lowry and Passonneau 1973	Fluorometric reading of NADP coupled reaction
Histo hemi l et i i g meth d		
Alkali etabl my sin ATP s	P dykula s d Herman 1955	Localisation of phosphatase activity towards ATP indicat d by $CoCl_2$
NADH diaphorase	Novikoff Shi nd Drucker 1961	Precipitation of diformazan granules
Gly g n synthet se	T k uchi and Glen r 1965	Demon tration of polysaccharide ynthesis by iodi tai ; G 6 P
Glycogen (PAS)	P ars 1961	Demonstr tion of glycol groups by periodic acid Schiff reaction
Q titati determin ti ns		
Glycog (6 %)	Lowry et l 1964 as modi fi d by Karlsson 1971	Fl orometric readings of NADP co pled reactio s
Oxyg pt k (submax 2 %; ma 3 %)	Haldane nd Priestley 1935	Dougl s bag method; Haldan tech ique

[x] Enzyme C mmissio number

obtained from a deep muscle site The standard sampling site used was 12 16 cm above the patella and 4 cm into the muscle There are sever l reasons for using the lateral portion of the thigh for muscle biopsies in addition to the absence of any larg vessels or nerves in th region Glycogen breakdown and blood flow in this muscle as well as muscle temperature have been shown to be related to the bicycle work load performed (Grimby Häggendal and Saltin 1967 Hermansen H ltman and Saltin 1967 Saltin Gagge and Stolwijk 1968) More recently Henriksson nd Bonde Petersen (1974) found a rectilinear relationship bet we integrated EMG activity a d a erobic power over a wide ra ge of work intensities

Weighing of amples Samples for q antitative enzyme determinat ions were immediately weighed on a C hn lectro balance (this procedure was completed within 3 min) and corrections were made for water evapor tion (extrapolation to zero time) Succinate d hydrogenase (SDH) activity was determined o fresh muscle tis The sampl s for determinati of glycogen synthetase (GS) and h okinase (HK) acti ity w re frozen in liquid nitrogen and stored at 80° C Determinations were mad within 2 days No ch nges caused by this proced re were fou d in the ctivity of these enzymes wh n comp risons were m de with determinations sing fresh mu cle hom g ate

Sampl s for qu ntati d t rmi tions (glyo gen) were alw ys immedi t ly fro in liq id nitroge while either till in th e dl or after having bee remov d with a forceps N ch nges i glyo g conte t were b erved withi 10 mi at room tempera ture (H ltma 1967) Sampl were stored at 80° C ntil n lysed

M ti g f sampl s for histo hemic l an lysi was performed aft r orie ting the fibre that tran vers s ction o ld be ut The mou ting medium sed was Ame Tissu T k The freezing procedure was u d rtake in liquid nitrog n or is p ntane cool d in liquid nitrog The latter proced re was used to void fr eze rtef cts i th tiss e Liquid itrog n is k ow to pr duc a gas layer aro nd the ample th reby i hibiti g immediate freezi g

throughout the entire muscle sampl Samples were alway c t in a cryostat at 20° C

Subjective rating of PAS stai ing intensity A rating system f r PAS staining int nsity was used in order to demonstrate glycogen distribution in muscl fibres The rating system compri ed four diff rent cat gori s d rk (th fibre most darkly stained for PAS in the sample) mod rat light a d negative (o taining) R ti g always took place u der the light micro scope directly from PAS tain d slide Microgr phs of ample st ined f r myo i ATPas w re u d to id tify the fibre typ s The reliability of the rating method was e al ted i g a photometric method (Edgerton et l 1974) Th correl tion ratio f th bjective evaluation of PAS staining i t ity to th photometric re ding was found to be 77 F r further dis u sio ss ref V Th proc dure described b s is the one s ggested by Stein and Padykul (1962) d similar to th u ed by Kug lberg and Ed tröm (1968)

RESULTS

Cl ifi ti f h ma kelet l mu cl fibre (I)

Tw fibre typ w re identifi d o th b is of st ining i t ity for myosi ATPa The tw fibre typ w re desig ted low twit h (ST) a d f st twit h (FT) ST fibres displ y d low tain i g i t ity f r my i ATP s nd FT fib were th revers F thermore ST fibre w re more i t ns ly t i d for NADH di phor the FT fibre Th h ract ri ti t i i g p tterns re hown i Fig 1 (l t p ge) No third di ti t fibr typ a f nd i p ies th r th n m w bserved Thi d t ma th t fibr di pl ying b th high myo i ATP s nd NADH di ph r st ining i ten ity wa n Th t i i g i t ity f r NADH di ph ra in FT fibr s ri d triki gly from ubject to bj ct b t t within th ame mu cl S cin t d hydrog na tivity w gre te t i th m t d rena w ll tr i d mu l p i ting to sp cifi l liz d trai i g ff t

Fibre distribution: Wide inter-individual variations in fibre composition were found in both well trained and untrained subjects (I) However variation between the two muscles studied in the same subject seldom exoe ded 10 15 % The coefficient of variation was 4 6 % This value was based on repeated sampling from the same muscle portion or from the opposite extremity The proportion of ST fibres was most often found to be greate t in muscl s from subj ts who participated in end ra ce activities whereas strength trained subjects w re the same as sedentary subje ts It is w rth noti g that this latter group only contai ed one successful athlet and he had 74 % FT fibres

Fibre are The area of FT fibres wa larger than the area of ST fibres in most subjects Both fibre types appeared to be larger i the legs than in the arms of most subjects There was some indic tio that type of trai ing also aff cted fibre rea in trained subjects making exte sive use of their arms (canoeists and swimmers) r legs (bicyclists) Despite this a li e r rel tionship w still fo nd betwee th percentage of ST fibre a d th relati area tak n up by them Thu the compositi n of a muscle in terms of the p rcent ge of fibre types is a good indication f th rel tive rea contributed by e ch fibre type t the tot l mu le

Gly og stor ge i mus l fibres (I II III a d IV)

Evalu t d o the basis of PAS staining int n ity no niform pattern for the t rage of glycog app ar to exist in th two typ s of k let l muscle fibres (I II III IV) However FT fibre w re more darkly tain d for glycogen tha ST fibres when glyc g nte t of th muscle wa less th 80 mmol glu ose ite kg [1] (II III) Staining int nsity was t a maximum whe gly og content exc eded this l v l It was there fore difficult to determi wh ther r not th re were a y bo io diff re es in glyo ge conte t b twe n th fibre types This w s furth r bste tiat d by th fi ding of a PAS taini g i t nsity in ome ST fibre which w e high r high r th n i FT fibre

in the cross ectional study (I) It was also obvious th t glycogen content was greater in muscles habitually sed to the greatest e tent

After previou glyc gen depl tio by me ns of e ercise the resy thesis process was rel tively slow d spite a carbohydr te enriched di t and without phy i al activity as far as pos ible (III IV) Almost 46 h were needed to reach pre ex rcise glycogen levels Synthesis occurred at a faster rate i the first 5 10 h after depletion PAS taining indicated a slight t dency for FT fibre to resynthe ize th ir glycogen more qui kly than ST fibres The p rc tage of the enzyme glycoge sy th tase prese t in the a tive I form incre sed when glycogen conte t wa low d a gradual d line occurr d with risi g glycogen l v ls (IV) Enha ed glycog n a cumulation al o occurred de pite a low l vel for the I form The taining intensity f r total glycoge y th tase (I D) reveal d difference b twee the two fibre types Howev r ST fibre were more darkly tained for th I f rm aft r depletio indicating an e hanced y the i ap ity b t thi uld n t b related to any greater i reas i PAS stai ing intensity

Ch g i rb hydr t (CHO) c t nt of th di t altered mus le gly ge l el (II) Gly g l vel w re low (me n 43 mm l glu e u its x kg [1]) with a f t d prot i di t a d FT fibre were more d kly t i d f r glyc gen than ST fib Aft r mixed a d CHO enrich d di ts glyo g le ls were high (m a 84 a d 144 mmol gl os u it x kg [1] re p ctiv ly) and differe c in PAS t ini g intensity ld be n b twe n th fibre typ

I flu e of tr i i g (IV) A omp ri o of tr i d d tr i d l g mu les from th am ubj cts di cl d ig ifi a tly high r glycogen level in tr in d l g th n in trai d l g A high r le l f tivity f r the ym glycog y th t (I D) a d he oki as w f d i tr i d l g N incre sed r t of gly g ynthesis w t d i tr i d mus les

Glycog n d pletion in muscle fibres (II V)

Glycogen depletion occurred preferentially in one fibre type or the ther d pending on exercise conditions (V) At varying work i tensitie up to work loads requiring $\dot{V}O_2$ max ST fibres always were the first to lose PAS staining If work continued some FT fibres were also depleted The rate of depletion increased with incre sing work i ten ity The glycogen still remaining in the muscle fter exercise at low intensities (non exhaustive) was locat d in both ST and FT fibres but whe exercise h d been performed t high intensities (leadi g to exhaustion) glycogen was found ex lusively in the FT fibres At supramaximal work load (exc eding $\dot{V}O_2$ max) which could not be su tai ed for con tinuous peri ds longer than 1 5 min FT fibre lost glycogen first or at the same time a ST fibres (V) There were always som empty FT fibre A y gly ogen still remaining after such exercise was fo nd i both fibre types When the pedalli g rat wa vari d ther by rying the rate of contraction no change w s en i this glycogen depletion pattern (V)

A somewhat different glyc gen depleti pattern w s revealed wh n almo t the me rel tive work was performed on a bi ycle rgometer with v rying i itial muscle glycoge levels (II) Pre e rcise glycoge levels were low after a fat nd protein diet (mean 43 mmol gluco u its x kg [1]) PAS staining di ol s d no darkly st in d ST fibres wher as most fibres were lightly st i d FT fibr s were more d rkly st i ed o erall than ST fibr (17 % dark and th majority moder tely tain d) D ring erci th red ti in mu le glycog n l v ls (PAS staining inte ity) o curred prefer tially in FT fibres More FT fibres th ST fibre wer gativ ly st ined f r gly og n following exercise Aft r mi ed nd CHO-e ri hed di t me n muscle glyc g l l amo nt d to 88 and 144 mmol glu o its x kg [1] respe ti ly At thi tim th int nsity of PAS stai i g w s dark and similar in both f bre type W rk produ d prefer nti l lo s f PAS t ining i ST fibres und r th se two diet ary c nditi Som FT fibre w re al depl ted aft r e ercise when starti g at the lower glycog n l vel Thi w s not the case

when starting on the highest glycogen level In both cases no fibres were negatively stained for glycogen following exercise and residual glycogen was found in both fibre types

GENERAL DISCUSSION

Cl ssificatio of human skeletal muscl fibres

Muscle fibres have been classified in many different ways (see Table I) The use of the terms slow twitch and fast twitch (ST a d FT) is based on the contractile characteristics of the fibres Bárány reported that the specific activity of myosin ATPase is c rrelated to th speed of muscl contr ctio (Bárány 1967) In addition Guth and Samaha (1969) demonstrated that actomyo i ATP se ctivity is correlated to histochemically demonstrable myosin ATP se The histochemical bi chemical and c ntractil properties of mus le fibres h ve be analysed in g in a pig mu cle A slowly contr cting muscle was then shown to correspond to a low le el of myosin ATPase activity as demo str ted by both qu ntitative a d hi t chemi l m ns (B rn rd et al 1971)

Ass ming th t thes results are pplicable to human tis u obvi u ly prese t c rtain difficulti s How r some reports hav been publish d suggesti g th t hum mu cle is compos d of tw fibre types based n diff re s in twit h contr ction time (Eb rst in a d Goodgold 1968 M Comas d Thomas 1968) Buchthal a d Schmalbruch (1970) orrel ted differe ce i c ntractio rate to high d low myo in ATPa at i i g i t iti s Furth rmore recent re ults o i terc t l muscl indi at a correl tion b tw n contr cti n time nd qu ntit tiv ly me ured myosin ATP se tivity (Edg rt n p rs l ommu ic tio) which is in g d agreement with dat o the human fl or muscl (Bárány 1967) These data provide s pport f r th se f th terms slow twitch nd fast twit h mu cl fibre v n for human mu cl tissue

Th re so f r not si g n c id tiv taining s basis for cl ifi ati n is th t conti um of staining int n ities from

darkly to lightly stained fibres is revealed in both ST and FT fibres This overlapping makes it difficult to distinguish between fibre types Moreover the oxidative pote tials of the muscl cell is increased by training thereby changing the taining pattern and the basis for classification (Holloszy 1967 G llnick et al 1973 a) No such effect on myosin ATPase following physical exercise ha yet been reported (Barnard Edgerton and Peter 1970 Gollnick et al 1973 a) even though this effect has been noted i a imal following cross innervation and electrical timulation (Close 1965 Dubowitz 1967 Bárány and Close 1971 Strèter et l 1973 Brown et al 1974) No data on the effects of training on myosin ATPase ctivity in man are available It seems rea onable t assume that contractile characteristics are relatively stable as compared to metabolic properties therefore providing suitable basis for fibre classification

On th b is f the pre ent dat some types of tr ini g are apparently capabl of i d cing preferential enlarg ment of ither fibre type (I Edström and Ekblom 1972 Gollnick et al 1973 a) A lin r relatio ship till exist d b twe n ST percentage distributio and relative are I view f the larger area of FT fib re in muscles from weight lifters and sprinters a larger numb r of s oh bjects might po sibly alter the shap f the distribution curve These ategories repres ted only 5 % of the popul tion e amined i th pre nt st dy

F tors related to glycog toras

Vari ti i tot l gly oge ont nt were f und betw en muscl s and subj cts b t ot within differe t p rtions of the same mu cle (Hultman 1967) Normal al s of abo t 1 2 g x 100 g wet mu l h ve been reported in the quadricep fem ris (H ltman 1967) but somewhat higher values are usu lly found i trained s bjects (IV Hermans n Hultma and S lti 1967) Resynthesis of glycogen i human skel tal muscle following xer ise depleti n h s previou ly b n analys d (B rg tröm and H ltman 1966 B rg tröm Hultman a d Roch Norlund 1972) Ac ordi g to these tudies glyc g n levels ven greater than the val es noted prior to depleti n were found

in muscle after only one d y on a carbohydrate enriched diet These results were not confirmed by the present data (for further discussion see ref II and III) The highest rate of synthesis observed in this study was 8 4 mmol glucose nits x kg 1 h 1 Compared to a glycogen breakdown of arou d 30 mmol glucose nits x kg 1 x h 1 in exercise at work load demanding about 60 70 % of $\dot{V}O_2$ max which could be performed for 2 h synth sis only amo nts to one third of the glycogen consumed in gly lysis

Glycogen synthetase h s th lowe t activity of th different enzymes inv lv d i th glycogen sy thetic pathw y and i there fore considered to be rate limiting Activity i regulated by the two int ro vertibl forms of the enzym th D form (depen dent for activity o th presenc of glucose 6 ph sph t) and the I form (independent of this activ t r) The conversi is c talyzed by phosphorylating and dephosphoryl ti g zyme Fac tors ff cting the activity f the nzymes regul te the ctivi ty f glycogen synthesis An invers rel tion hip b tween th glycog n content of the mu cl nd synth ta I f rm ctivity h b rep rt d (D f rth 1985) Thi i dicate regul ti g feed b ck mechani m by glycoge it lf In th pre t t dy the high st sy thet s I activity wa rev l d when th glycoge te t f th mu cle w low A gr du l de li w lso noted with incre i g glyc g n lev l How r gly oge d e n t app ar to b th nly reg l ting fa t r i c ST fibres d pite dark r st ining int nsity for th I form f il d to di play a higher r te f y th i Th tr p rt of gl c s into the mu l cell and its ph sphoryl ti c t lyz d by he oki e re al import nt to glycogen mulati Th high r h x kin e tivi ty f u d i tr i d mu l may i di te a gre t r upply f gl c 6 ph ph t whi h i b th n ctivat r and a ubstrat f r glycoge y th i A membr tr nsp rt w s n t v l t d i th pre t t dy d h b e d crib d previou ly it will not be t k p i th pre t di io (for ref s N rsh re nd C ri 1988)

The higher gly g l is rep rt d pre i usly i trai d mu cle n ow b rel t d t traini g and n t to di t adj stment Th bility t more th doubl gly og n co t nt the o lled

over-shoot or supercompens tion phenomenon still awaits expla nation Enhanced glycogen accumulation was noted despite low synthetase I activity It is obvious that the on and off mech nism for glycogen synthesis is a complex process affected by the other events in the cell

Factors related to s lective glycog n depl tio d ring e ercise

Glycogen breakdown during diff rent e ercise conditions has been widely examined Res lts were based on assays of muscle biopsy specimen witho t muscle fibre differentiation As indicat d by PAS stai ing exercise led to selective glycogen depletion in muscle fibres Thus true functio al changes may be underesti mated when evaluations are based on assay of mixed mu cl samples

The exte t to which the PAS staining technique provides an e a t answer is q estionable especially at maximal staining i t nsities PAS st ining appears to be at a maximum t glycogen levels e c eding 60 mmol gluco e units x kg [1] Changes i glyco ge content abo e thi level cannot be detected as ch nges in PAS t i i g inte sity th reby making misleading re ults possible If FT fibres contai more glycoge the ST fibres the e rly appe ranc of PAS neg tive ST fibre may be misl adi g sinc far mor exten iv gly g n br akdown lthough masked by the higher i itial glycogen cont nt may h o curred i FT fibres Glyco ge breakdown i ST fibres may lso h taken pl ce but imp ss ible to detect by cha ge in PAS st ining because of their gre t er oxid tiv capacity thereby e h ncing utilizati n of fat with a glycogen sp ring effect

If the hi tochemi l disappe ranc of glycog n (PAS st i ing intensity) from mu cl fibr s is employed to reflect tru hanges it may als b used to discuss th diff re ti l recruitme t of fibre Each motor u it is ge erally reg rded a being made p of ne typ of mu l fibr (Kugelberg a d Edström 1968) Th rel ti ship b tw en the ability to withst nd f tigu and hi to chemical fibre type h s b e demonstrated with lectri al stimu latio (K gelberg and Edström 1968) Fibres displayi g gre t staining inte ity f r o idative enzymes (red typ I r ST fibres)

were found to be less affected by electrical stimulatio and were able to ustain contractio s for longer period of time the white type II or FT fibres A more rapid lo s of glycog n (phosphorylase staining intensity) from type II fibre was also take as an indicatio f previo s a tive fibres (Kugelb rg and Edström 1968 Edgerton et al 1970 a) However the reverse t i ing pattern was reve led after physio l x rcise (Edgerton t al 1970 b Armstrong Shepherd and Gollnick 1973) This indicates th t ST fibres ar recruit d first during physiological a tivat ion circ mstance possibly scribable to a lower ctivation thresh ld for ST fibres (Henneman d Olson 1965) This kind f timulation may also possess a lower frequency th the stimu lati produced by l ctrical means so that th activatio thre hold of FT fibre i ot reached A work contin s ST fibres b ome gly gen depleted nd most probably u able t tisfy energy requirements FT fibre r then gr d ally re ruit d (V Gollnick et al 1973 b) A alre dy mention d tension lso pp rs to b an import nt factor in fibre re ruitment A hift i e rly gly g n los (PAS stai i g i tensity) from ST t FT fibres w s n when i ometri c tra ti ns c lli g for more th n 20 25 % f maximal volu t ry c ntra til tre gth w re p rf rmed (G ll i k et l 1974) How v r h c rrel tio co ld b fo nd d ri g bicycl ex rci e (V) At thi p int it may b worthwhil to mph si e th t th s l oti lo f glyc g from ST d FT fib res not d h re wa b ed b ervati from l g p ri d f rci (l ting for ral minut t h rs) At th t of x rci e FT fibres have b hown t b ctiv ted for a brief p ri d (Grimby nd H r 1988) Thi may l b the c s f r brief p ri ds l t r n i bm imal x rci

In ncl si th pre t t dy m y b id t ha how that o ly two maj r fibre typ re f d i th k l t l mu l f m a d th t th se two fibre typ s t i imilar amo nts of gly og n Gly og t r g d d pl ti may t k pl c t som wh t diff re t rat i th tw fibre typ t a rtain t t Di t wo k i t nsity a d th typ f ex rci re fa t rs f imp rt B th th m tab li d c t tile prop rti s f th mu l fibre pp a t c trib t t thi ph nome

ACKNOWLEDGEMENTS

This work was performed at the Department of Physiology at Gym astik och idrottshögskola Stockholm Economic and l bor tory facilities were placed to my disposal by the he d of the d partment Prof ssor Per-Olof Åstrand

I wish to express my deep gratitude to Professor Bengt Saltin nder whose guidance it has been my privilige to work Hi help advice a d never ceasing ide s have bee of inestimable valu

My sincere thanks lso to Dr Philip Gollnick for fruitful collaborati n a sistanc e d intere ting discussions a d also to the former head of th department Profe sor Erik Hohwü Chri te n who was of import nce to me whe writing the pre e t thesis

I also wish to th nk Drs Ja Karls o and Ste Adolf so for t chni al advic di c ssi s and critici m a d all other colleag e and frie ds at th Department f Physiol gy Gymnastik h idrottshögskola

The t di e w re pported by gr nts from th Sw di h Medical Re e rch C ncil (14X 2203 40X 2203 14V 4014) R se rch Council of the Swedi h Sports F deration (73 27) Semper Nutrit i n Foundation a d th W lle berg Fou dati

REFERENCES

ARMSTRONG R B R E SHEPHERD a d P D GOLLNICK Gly ogen depletion patterns in rat sk letal muscle at different xercise intensities J appl Physiol 1973 00 000 000

ÅSTRAND P O and B SALTIN Oxygen upt k d ring the first minutes of heavy mu ular exercise J appl Physiol 1961 18 971 976

BARANY M ATPase activity of my si o rrelated with p ed of muscle sh rtening J gen Physiol 1967 50 197 218

BARANY M and R J CLOSE The tra sformati of my si i cro s inn rvated rat muscles J Physiol 1971 213 455 474

BARANY M K BARANY T RECKARD nd A VOLPE Myo i of fast and low muscl s f th rabbit Arch Bi hem Biophy 1965 109 185 191

BARNARD R J V R EDGERTON and J B PETER Effe t f roi on skel tal muscle II Contractile prop rti J ppl Phy i l 1970 28 767 770

BARNARD R J V R EDGERTON T FURUKAWA and J B PETER Hi t hemic l biochemic l d o tra tile prop rties f red whit a d intermedi t fib rs Amer J Phy iol 1971 220 410 414

BERGSTRÖM J M scl l ctrolyte i m Sca d J li Lab Invest 1962 S ppl 68

BERGSTRÖM J nd E HULTMAN Mus l gly g y th i fte exerci e enh ci g f t r locali ed to th m scl ll i ma Nat re (L d) 1966 210 309 310

BERGSTRÖM J E HULTMAN nd A E ROCH NORLUND M s l glyc g y th t i rmal ubj t S nd J lin Lab I t 1972 29 231 236

BERNARD C D l matière gly gè con idérée omme nditi d d loppement d rtai ti s ch z l fo t t l pp riti d l f cti n gly gé iq d f i Compt r d A d S i 1859 48 80

ACKNOWLEDGEMENTS

This work was performed t the Department of Physiology at Gymnastik och idrottshögskolan Stockholm Economic a d laboratory facilitie were placed to my disposal by the he d of the department Professor Per-Olof Åstrand

I wi h to express my deep gratitude to Professor Bengt Saltin under whose guidance it has bee my privilige to work His help advice and ever ceasing ideas have been of inestim abl valu

My sincere thanks also to Dr Philip Gollnick for fruitful collaborati n assi tance and interesting discussions and also to the former h ad of the department Professor Erik HohwÜ Christensen who was of importance to me when writing the prese t the is

I l wish to th k

Drs Jan Karlsson and Ste Adolfsso for technical advice dis cu sions nd riticism and all other colleagues and frie ds at the D partment of Physiology Gymnastik o h idrottshögskolan

Th studie we e s pport d by gra ts from the w dish Medical Rese rch Co cil (14X 2203 40X 2203 14V 4014) R esearch C u il of th Sw di h Sport Fed ration (73 27) Semper Nutrit ion Found ti a d the W ll berg Foundation

EDGERTON V R R J BARNARD J B PETER D R SIMP ON nd C A GILLESPIE Response of mu cle glyc ge d ph phoryl to lectrical stimul tion i trained and ontrained gui e pigs Expl N rol 1970 a 27 46 56

EDGERTON V R D R SIMPSON R J BARNARD nd J B PETER Phosphorylase a tivity in ac tely xerci ed mus le Nat re 1970 b 225 866 867

EDGERTON V R B ESSEN B SALTIN nd D R SIMPSON Glycoge depl ti n in pecifi typ s of human skeletal muscle fibers in intermittent and contin ous erci I Met boli Ad pt ti n to Prolonged Phy ic l E ercise S co d I tern tio l Symp sium n Bi chemistry of Exercis 1974 I th pre s

EDSTRÖM L nd B NYSTRÖM Hist chemical typ s d s of fibres of normal human mu cles A ta eurol s d 1969 45 257 269

EDSTRÖM L d B EKBLOM Differe e i size f red d whit mu le fibres in va t s l teralis f m c l s q d p fem ris of n rmal individu ls and thletes R l ti n to phy ic l p rformance Sca d J cli Lab I st 1972 30 175 181

ENGEL W K Th enti lity of histo nd yt hemi l t di s of k l tal mus l i i vestigati f euromu l di s N rology 196 12 778 794

GAUTHIER G F D th rel ti hip of ltra tru t ral d yt chemi l fe tures t olor i mammali sk l t l mu l Z Z llf rs h 1969 95 462 462

GEORGE J C d R M NAIK Rel tiv di trib t e d h mi al n t re f th f l t re f th tw typ s f f b rs the p ctorali m j r mu cl f the pig Natur 1958 181 709 711

GILLESPIE C A D R SIMPSON d V R EDGERTON H gh gly ge tent f red pp s d to whit k l t l mu l fib re of g i s pig J Histo hem Cyt chem 1970 18 552 558

BOCEK R and C H BEATTY Glycogen synthetase and phosphory l se in red and white muscle of rat and rhesus mo key J Histochem Cytochem 1966 14 549 559

BROWN M M COTTER O HUDLICKA M SMITH and G VRBOVA Metab lic changes in long term stimulated fast muscl In Met bolic Adaptation to Prolo ged Physical Exercise Seco d I ternational Symposi m o Biochemistry of Exercis 1974 In the pre

BUCHTHAL F a d H SCHMALBRUCH Contraction times a d fibre types in int ct human muscle Acta physiol scand 1970 79 435 452

BURKE R E Mot r nit types of cat tric ps surae muscle J Physiol 1967 193 141 160

BUSK G and T HUXLEY (tr) [Kolliker s] Manual of Human Hi tology Lo d n 1853

CLOSE R Eff cts of cross union of motor nerves t fa t and slow skeletal muscl s N ture (Lond) 1965 208 831 832

COOPERSTEIN S J A LAZAROW and N J KURFESs A microsp ctro photometr method f r th determinati n of s ccinic d hydro ge e J bi l Chem 1950 186 129 139

DANFORTH W H Glycoge sy theta activity in sk l t l mus l J bi l Chem 1965 240 588 593

DENNY BROWN D E The histological fe tures f triped mu cle i relati t te f tional a tivity Proc roy Soc B 1929 104 371 411

DUBOWITZ V Cr s i rv ted mammalia k l tal mu cle histo chemic l phy i l g l and bi hemi al b ervation J Phy i l (Lo d) 1967 193 481 486

DUBOWITZ V d A G E PEARSE A compar tive hi tochemi al study f id ti e zym and ph ph ryl se activity i skel tal mu le Hi to hemie 1960 2 105 117

EBERSTEIN A d J GOODGOLD Slow and f st twit h fib re i human keletal mu cl Amer J Physi l 1968 15 535 541

EDGERTON V R R J BARNARD J B PETER D P I ■ C F
GILLESPIE Response of muscle glycogen and phosp cryla ■
electrical stimulation i trained a d normal cd g i ra tx
Expl Neurol 1970 a 27 46 56

EDGERTON V R D R SIMPSON R J BARNARD and J B PETER
Phosphoryl ae ctivity in acut ly ■ ercised m scle Na re
1970 b 225 866 867

EDGERTON V R B ESSEN B SALTIN and D R SIMP ON Gly g n
depletion in spe ifi types of human skeletal muscle thars
in i termittent and continuous ■ ercise In: Metabolic Adapta
ti n to Prolonged Phy ical Exercise Second I ternatio al
Symposium on Biochemistry f E ercise 1974 In th press

EDGTRÖM L nd B NYSTRÖM Hist chemical types o d sizes
fibres f normal human muscles Acta ne rol sca d 1gr 4
257 289

EDSTRÖM L nd B EKBLOM Diff renc ■ in sizes of red a d hit
mu cl fibre in v t ■ l t ralis of musculus quadriceps
femoris of ormal i divid l nd athlet ■ Relation to
phy ical p rform n Scand J lin L b In est 1972 30
175 181

ENGEL W K Th ■ tiality f hi t and ytochemical studies
of kel t l mu cl in i vestigation of uromuscular disease
Ne rology 1962 1 778 794

GAUTHIER G F O th relati nship f ultrastructural a d cy
chemic l f ture t olor in mammali n skeletal muscle Z
Zellf rsch 1969 95 462 482

GEORGE J C d R M NAIK R lativ di trib ti n a d che ical
nature f th f l t re f the tw types f fibers i the
pe t rali maj r mu l of th pig on N tur 1958 181
709 711

GILLESPIE C A D R SIMPSON nd V R EDGERTON High glycoge
o t t of red a pp ■ d t whit kel tal muscl fibe ■ of
g i a pigs J Hi t h m Cyt chem 1970 18 552 558

SALTIN B A P GAGGE AND J A J STOLWIJK Muscle temperature during submaximal exercise in man J appl Physiol 1968 25 678 688

SASKI M and T TAKEUCHI Histochemical and electron microscopic observations of glyo gen synth tized from glucose 1 phosph te by phosphorylase nd branching enzyme in human muscle J Histo chem Cytochem 1963 11 342 352

STEIN J M and H A PADYKULA Histochemical classification of individual skeletal muscle fibers f the rat Amer J Anat 1962 110 103 124

St GEORGE STUBBS S a d M C BLANCHAER Glycogen phosphoryla e and glyoogen synthetase activity in red and white skel tal muscle of the g inea pig C nad J Bioch m 1965 43 463 468

STRETER F A J GERGERLY S SALMONS nd F C A ROMANUL Synthesi by f t muscle of myosin light chains haract rist ics of low muscl in response to long term stim l tion Natur New Bi l gy 1973 24 17 19

TAKEUCHI T nd G G GLENNER Hi t hemical d monstratio of ridine diphosph t gl ose glycogen transf r se i animal ti Canad J Biochem 1965 43 463 468

THOMAS J A K K SCHLENDER a d J LARNER A rapid filter paper as y f r UDP gl o gly oge gl o yltransfer e incl di g an improved bio ynth sis of UDP ^{14}C glucose A lyt Biochem 1968 25 486 499

VILLAR PALASI C M ROSELL PEREZ S HIZUKURI F HUIJIWG a d J LARNER M scle d li er UDP gl c α 1 4 glucan α 4 glucosyltr n fera (glyc g synth t se) I M th d in E zymol gy Ed Neufeld E and V Gi berg A ad Pre s New York 1966 Vol 8 374 384

SALTIN B A P GAGGE AND J A J STOLWIJK Muscle temperature during submaximal exercise in man J appl Physiol 1968 25 679 688

SASKI M and T TAKEUCHI Histochemical and electron microscopic observ tions of glycogen synth tized from glucose 1 pho phate by phosphorylase and branching enzyme in human mu cle J Histo chem Cytochem 1963 11 342 352

STEIN J M and H A PADYKULA Histochemical classification of individu l skel tal muscle fibers of the r t Amer J Anat 1962 110 103 124

St GEORGE STUBBS S and M C BLANCHAER Glycogen phosphorylase and glycogen synthetase activity in red and white skeletal muscle of the guine pig Ca ad J Biochem 1965 43 463 468

STRETER F A J GERGERLY S SALMONS and F C A ROMANUL Synth sis by fast muscle of myosin light chain characterist i s of slow muscle in re ponse to long term stimulatio Natur New Biology 1973 24 17 19

TAKEUCHI T and G G GLENNER Histochemical demonstration of ridine dipho phate glucose glycog transferase in animal tissu Canad J Biochem 1965 43 463 468

THOMAS J A K K SCHLENDER d J LARNER A rapid filter paper assay f r UDP gluco e glyo ge glucosyltransferase includi g an improv d bio y thesis of UDP ^{14}C glucose An lyt Biochem 1968 25 486 499

VILLAR PALASI C M ROSELL PEREZ S HIZUKURI F HUIJING and J LARNER M s le d liver UDP gl os α 1 4 glucan α 4 glucosyltran fer se (glycogen synthet) In Methods i *Enzymology Eds Neufeld E d V Gi sberg A ad Pres* New York 1966 Vol 8 374 384

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 403

FUNCTIONAL ASPECTS OF 5-HYDROXYTRYPTAMINE TURNOVER IN THE CENTRAL NERVOUS SYSTEM

BY
KJELL MODIGH

GÖTEBORG 1974

tenstryckeriet AB Stockholm 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 403

FUNCTIONAL ASPECTS OF 5-HYDROXYTRYPTAMINE TURNOVER IN THE CENTRAL NERVOUS SYSTEM

BY
KJELL MODIGH

GÖTEBORG 1974

Printed [illegible]

CONTENTS

INTRODUCTION 5
Di tribution of central monoamine neurons 6
Synthesi of brai 5-HT 6
Storage release and inactiv tion 7
Regul tory mechanisms 8
Objectiv of the present tudy 10

METHODOLOGY 11
Subjects 11
Substances used 11
Motor activity 11
Spinal transections 12
Biochemical determinations of monoamines thei precursors and catabolites 12
Model used for the estimation of synthesis of transmitter and of impulse activity in entral monoamine neurons 13

STUDIES ON THE ROLE OF 5-HT IN BEHAVIOURAL EFFECTS OF ANTIDEPRESSANT DRUGS 15
Experiments with 5-HTP 16
Experiments with ni lamide 19
Experiments with L tryptophan 21
Tryptophan plus metabolic inhibitors 21
Tryptophan plus chlorimipramine 23
Clinical and physiological implications 23

STUDIES ON INTERRELATIONS BETWEEN NERVOUS ACTIVITY AND SYNTHESIS OF TRANSMITTER IN 5-HT NEURONS 27
Experiments with chlorimipramine and PCPA 27
PCPA 31
Stress experiments effects of isolation and fighting on 5-HT 32
Effects of isolation and fighting on CA i brai 34
Clinical and physiological implications 35

SUMMARY AND CONCLUSIONS 36

ACKNOWLEDGEMENTS 40

REFERENCES 41

The present thesis I mainly based upon the following eight esearch papers.

I Central and peripheral effects of 5-hydroxytryptophan on motor acti ity in mice Psychophamocologia (Berl) 1972. 23. 48-54

II Effects of chlorimipramine and protriptyline on the hyperactivity induced by 5-hydroxytryptophan after peripheral decarboxylase inhibition in mi e J Neural Transmission 1973. 34 101 109

III On the role of central nervous system catecholamines and 5-hydroxytryptamine in the I lamide-induced behavioural syndrome Br J Pharmacol 1972. 46 32-45 (together with T H. Svensson)

IV Effects of L tryptophan on motor acti ity I mic Psychopharmacologia (Berl) 1973 30 123-134

V Effects of p-chlorophenylalanine and monoamine oxidase inhibitor on the 5-hydroxytryptamine I th spinal cord after transection J Neural Transmission 1972. 33. 211 222. (together with N E Andén)

Effect of chlorimipramine on the rate of tryptophan hydroxyl tion I the I tact and transected spinal cord J Pharm Pharmacol 1973. 25 926-928.

VII Effects of isol tion and fighting I mice on the rate of synthesis of noradrenaline dopamine and 5-hydroxytryptamine I the brai Psychopharmacologi (Berl) 1973 33. 1 17

VIII Effects of social stress on the turnover of brai catecholamines and 5-hydroxytryptamine i mice Acta pharmacol t toxicol 1974 (I press)

These papers will be referred to by thei Roman numeral

INTRODUCTION

An Indol deriv ti e with pronounced timulatory effect on smooth muscl was Isol ted from enterochromaffi ell by Erspamer i 1946 (for eview see Erspamer 1954) Shortly thereafter Raport Green and Page (1948) isolated a compound from platelets which due to its asoconstrictor properties was named serotoni Later the compound isolated by Erspamer was shown to be identical with serotoni Its chemical tructur 3-(2-aminoethyl)-5-hydroxyindol (5-hydroxytryptamine; 5-HT) was determined by Raport (1949) 5-HT was l ter shown to be present in brai where it was found to be unequally distributed (Twarog and Page 1953; Ami Crawford and Gaddum 1954) as were the two catecholamines (CA) noradrenaline (NA) (Vogt 1954) and dopamine (DA) (Carlsson Lindqvist Magnusson and Waldeck 1958; Bertler and Rosengren 1959 Carlsson 1959; Bertler 1961) By means of the histochemical fluorescence technique i troduced by Hillarp and co-workers (Falck Hillarp Thieme and Torp 1962; Carlsson Falck and Hillarp 1962) these three monoamines were shown to be localized intraneuronally each of them i separate neurons withi the central nervous system (CNS) The highest concentrations were found i nerve terminal enlargements the so-called varicosities

The suspicion soon arose that these three monoamines may serve as transmitters i the CNS and there i now considerabl evidenc i support of this iew (see g. Carlsson 1967; Andén Carlsson and Häggendal 1969a) The first tentativ suggestion that brai 5-HT may hav an important rol in the control of mental function was based on the early findings that the hallucinogenic drug d-lysergi acid di thylamide (LSD-25) antagoni ed the effect of 5-HT on smooth muscl (Gaddum 1954); and that reserpine drug with pronounced antipsychoti and behavioural depressant effects lowered the concentration of 5-HT i the brain (Brodi Pl tscher and Shore 1955) These reports were among the most important i timulating further research on brai monoamines and thereby leading to the development of modern psychopharmacology However i spite of extensive studies during the last two decades the functional rol of the 5-HT neurons is till unclear (for review see Chase and Murphy 1973) A major hypothesis supported by indirect evidence is that 5-HT neurons may be involved i the control of mood (see g Coppen 1967; Kielhol 1968; Roos and Sjöström 1969; Carlsson 1970; Sjöström and Roos 1972).

In this summary of our esearch correlating behaviour with the 5-HT turn-

over I the CNS and investigating some of the mechanisms which regulate this turnover some data relating to the rol of DA and NA are also included A brief introductory review of some of the current concepts concerning the distribution and physiology of the monoamine neurons I bral I first presented

Di tribution of entral monoami neurons

The monoamine neurons I the CN have been mapped out both by means of the above-mentioned histochemical fluorescenc technique and by lesion experiments The cell bod'es of the 5-HT contai ing neurons are locali ed mainly I the different raphe nucl I I the brai stem (Dahlström and Fuxe 1965 Heller and Moore 1965; Andén Dahlström Fuxe Larsson Olson and Ungerstedt 1966) Axons project from these nucl I to irtually ll areas of the brain including for exampl the ticular formation hypothalamus triatum limbic forebrai and neocortex. Descending axons project to the grey matter at all level of the spinal cord the lumbar and sacral parts being particularly rich I 5-HT terminals (for detail see Fuxe Hökf lt and Ungerstedt 1970; Dahl tröm Häggendal and Atack 1973)

The majority of the CA neurons also originate from the brai stem Th NA rons project to widespread areas of the brai and to the spinal cord The DA project mai ly to the neostri tum and to parts of the limbic system (for detailed description see Fuxe t al 1970 Ungerstedt 1971)

Synthesis of brai 5-HT

Th synthesis of 5-HT begins with the hydroxyl tion of L tryptophan to 5-hydroxytryptophan (5-HTP) which in turn is decarboxyl ted to 5-HT (Udenfriend Titus W issbach and P terson 1956; Udenfriend and W issbach 1958) The conersion of L tryptophan to L 5-HTP catalyzed by the enzyme L tryptophan-5-monooxygenase (tryptophan-hydroxylase) I considered to be rate-limiting (Jéquier Lovenberg and Sjoerdsma 1967) The enzyme utili es oxygen as co-substrate and tetra-hydropteridine as an lectron source The presence of tryptophan hydroxylase in brai (for recent eview see Lovenberg 1973) was first demonstrated by Grahame-Smith (1964) This enzyme appears to be specifi for L tryptophan and to be located exclusively in th 5-HT neurons (see g Grahame-Smith 1967; Aghajanian

and Asher 1971; Kuhar Roth and Aghajanian 1971) It is presumably synthetized i the ell bodies of the raphe nucl i from where it is slowly transported (a few mm per day) to the nerv terminals by an axonal flow (Meek and Neff 1972)

L 5-HTP decarboxylase (for review see Udenf iend Christenson and Dairman 1973) appears to be identical with an enzyme capable of decarboxylating all naturally occurring aromatic amino acids Consequently the name aromatic L amino acid decarboxylase (DC) has been proposed (Lovenberg W issbach and Udenf iend 1962) This enzyme occurs in the peripheral and central nervous system as well as in extraneural tissues g in the kidney li er and i testine (for review see Hagen and Cohen 1966) It also occurs i the endothelial cell of the br in capillaries wher it constitutes partial barrier between blood and brai for the monoamine precursors 5-HTP and 3 4-dihydroxyphenylalanine (DOPA) (Bertler Falck Owman and Rosengren 1966; Bartholi i Constantinidis Tissot and Pletscher 1971) The regional di tribution of DC i the brain (i e "beyond" the blood-brai barrier) closely resembles that of the monoamines 5-HT DA and NA (Bogdanski W issbach and Udenf iend 1957; Kuntzman Shore Bogdanski and Brodie 1961 McCaman, McCaman Hunt and Smith 1965; Lloyd and Hornykiewicz 1972) Recently the DC i the mesencephalon has been demonstrated immunohi tochemically to be located i DA- and 5-HT contai ing cell bodies but not i ther nerv cell bodies (Hökfelt Fuxe and Goldstei 1973) Furthermore lesion experiments eveal that DC i the spinal cord withi the blood-brai barr er is selectiv ly located i NA- and 5-HT neurons (Andén Engel and Rubenson 1972) Together these findings indicate that the DC within the blood-brai barrier is located only i the monoamine neurons

The ynthesi of CA (for review see Blaschko 1973) proceeds from L para-tyrosine whi h is hydroxylated to form L DOPA. Thi tep catalyzed by L tyrosine hydroxylase (see g Udenfriend 1966; W i er 1970) normally appears to be rate-limiting i the ynthesis of both DA and NA The DOPA i decarboxyl ted to DA. In NA neurons DA is β-hydroxylated to NA. The DOPA-decarboxylase appears to be identical with the 5-HTP decarboxylase (vide supr)

Storage, lease and Inactivation

The newly-formed transmitter amines synthetized i the monoaminergi neurons ar largely taken up and tored i granules thereby being protected against intra-

neuronal catabolism (vide infra) The granular torage of these amines is probably essenti l for the release of transmitter induced by nerve impulses (see e g Malmfors 1965; Andén et al 1969a; Häggendal and Malmfors 1969- W iner 1970; Dahlström et al 1973; Smith 1973)

Monoaminergic transmitters released from nerv terminals in the CNS are believed to acti ate receptors of the postsynaptic ffector cells Apparently these hypothetical receptors have high degree of substrate specificity: thus g 5-HT eceptors are not activated by DA and vi e ersa (Andén 1968)

The action of monoaminergi transmitters at the eceptors is terminated by catabolism outside the monoaminergi nerv terminals and probably of more importance by acti re-uptak of the transmitters into the presynaptic nerv terminal followed by catabolism or re-uptake into granules (for review see Andén t al 1969a; Carlsson 1970; Iversen 1970) The active e-uptake mechanisms i e. the membrane-pumps mentioned abov show certai degree of substrate specificity towards thei normal transmitter i the different monoamine neurons: thus 5-HT i low concentrations (~0 1 µM) is preferentially taken up by the membrane pump i 5-HT neurons. Thi el tiv specifi ity i however lost at higher concentrations of transmitters g high concentrations of 5-HT are taken up i to CA neurons (for eview see Snyder Shaskan and Kuhar 1973)

5-HT is chiefly catabolized both inside and outside the 5-HT neurons to monoamine oxidase (MAO) to 5-hydroxyindole-3-acetaldehyde which i turn i converted to 5-hydroxyindole-3-aceti acid (5-HIAA) i an ldehyde dehydrogenase (Titus and Udenfriend 1954; Sjoerdsma Smith Stevenson and Udenf iend 1955; for review see Blaschko and Levine 1966) MAO i widespread in peripheral tissues and i the CNS where it i intracellularly bound to mitochondria. Both glial cell and nerve cell contain MAO (for review see Tipton 1973) Th i traneuronal catabolism of CA also occurs mai ly i MAO However CA are also catabolized i the extracell lar space i catechol O-methyltransferase (COMT) (for review see Andén t al 1969a; Jonason 1969- Sharman 1973)

Regulatory mechanisms

The metabolism torage and release of 5-HT i the CNS seems to be egul ted by several different mechanisms The natur of these mechanisms i only partially elucidated

The tryptophan hydroxylase is not normally saturated with tryptophan (Eccleston Ashcroft and Crawford 1965 Fernstrom and Wurtman 1971; Grahame-Smith 1971; Carlsson and Lindqvist 1972) Consequently the rate of 5-HT synthesi depends both on the concentration of tryptophan i 5-HT neurons and on the activity of tryptophan hydroxylase

L Tryptophan i taken up into br i and synaptosomes by means of carrier mechanisms Several ther neutral amino acids compete for these uptake mechanisms (Guroff and Udenfriend 1962, Blasberg and Lajtha 1965, Grahame-Smith and Parfitt 1970, Kiely and Sourkes 1972) Consequently the uptak of tryptophan i to the brai i dependent on the relation between the plasma concentrations of both free tryptophan (i not bound to albumi) and of other neutral amino acids (Fernstrom and Wurtman 1972) A study of the uptake of tryptophan i to only 5-HT neurons has not been possible thus it is not known whether thi uptake differs from that occurring i other cell withi the CNS

The tryptophan hydroxylase acti ity seems to be partially dependent on the flow of nerve impulses i the 5-HT neurons Support for thi iew is provided by the findings that the acti ity of tryptophan hydroxylase was moderately decreased i acute lesion experiments (Carlsson Bédard Lindqvist and Magnusson 1972a, Carlsson Lindqvist Magnusson and Atack 1973b, Schubert 1973) and increased during the electrical timul tion of raphe nuclei (Shields and Eccleston 1972) Sinc these changes i enzyme acti ity are rapid they probably reflect different degrees of activation of the enzyme rather than variations i the synthesi of new enzyme

Tryptophan hydroxylase acti ity has also been proposed to be regulated by end-product inhibition i by ariations i the i traneuronal concentration of 5-HT (Macon Sokoloff and Glowinski 1971; Hamon Bourgoi and Glowinski 1973 Schubert 1973) Results from i itro experiments are however contra-di tory i thi espect (cf Grahame-Smith 1964 McGeer and P ters 1969 Jéquier Robinson Lovenberg and Sjoerdsma 1969 Hamon t al 1972)

At least under ertai conditions part of the 5-HT seems to be cataboli ed i traneurally without having been functionally activ This iew is supported by esults from behavioural experiments (Grahame-Smith 1971) and lesion experiments (Bédard Carlsson and Lindqvist 1972) Thus at least two different compartments for newly-formed 5-HT may exi t one functionally active and th other functionally

inactiv (cf Grahame-Smith 1973) Such a compartmentali ation may well hav the regulatory function of compensating for ariations i the rate of 5-HT ynthesis which occur independently of the functional need for transmitter

Drugs which by different modes of action are supposed to acti ate central 5-HT receptors depress th firing i raphe cells Thi inhibitory effect may well be mediated i a feed-back mechanism which is ctivated by an increased stimul tion of pre- or postsynapti 5-HT eceptors (for eview see Aghajanian and Haigler 1973) Such feed-back mechanism may serve the purpose of counter acting the change in acti ity f the 5-HT receptors)

Objectiv of the present tudy

Drugs with antidepressant properties and well documented effects on the synthesis or inacti tion of 5-HT wer examined Attempts wer made to evaluat thei effects at the 5-HT receptors by means of the behaviour l changes whi h they induce i animal Furthermore the possible rel tionship between the impulse activity i 5-HT eurons and the rate of ynthesi of 5-HT was examined The study has implications for cli ical research on the possibl involvement of central 5-HT neurons i mental diseases Major approaches used i such linical research are: 1) to evaluate the therapeutic effects of drugs which by different modes of action i terfere with the neurotransmission in 5-HT neurons; knowledg of the effects of these drugs t the 5-HT eceptors i then of fundamental importanc 2) to search for correl tions between me tal symptoms and changes in the rate of turnover of 5-HT in the CNS the ignificance f such hanges i turnover depends on the tent to which they reflect the funct onal activity i th 5-HT neurons

METHODOLOGY

The methods used i the experiments ar described in detail in the appropri te papers. A brief survey of these methods is gi en below

Subjects

Male mice (NMRI strain) weighing 20-25 g and mal Sprague-Dawley rats weighing 180-220 g wer used

Substances used

L Tryptophan (Ajinomoto Co Inc. Tokyo)

DL 5-Hydroxytryptophan (Ajinomoto Co. Inc Tokyo)

DL-α-Methyl-metatyrosine (α-MMT Regis Chemical Co. Chicago)

Chlorimipramine HCl (Ciba-Geigy Basel)

Protriptyline HCl (Merck Sharp & Dohme International Haarlem)

Nialamide HCl (The Swedish Pfi er Ltd Näsbypark)

Reserpine (The Swedish Ciba Ltd. Stockholm)

DL p-Chlorophenylalanine methylester HCl (PCPA); (H69/17; Hässl Göteborg)

DL-α-Propyldopac tamide (H22/54; Hässl Göteborg)

DL-α-Methyl-p-tyrosine methylester HCl (α-MT); (H44/68 Hässl Göteborg)

L-α-Hydrazino-α-methyl-β-(3 4-dihydroxyphenyl) propioni acid (MK 486; Merck Sharp & Dohme International Haarlem)

3-Hydroxybenzyl hydrazine HCl (NSD 1015 Smith & Nephew Research Ltd Harlow)

5-Hydroxypyrazolo-(3 4-d)-pyrimidine (Allopurinol; Burroughs W llcome & Co. London)

All drugs wer injected i approximately neutral sol tions by the intraperitoneal route

Motor activity

The motor acti ity was in our earlier experiments measured by means of Animex activity meters (Farad Inc Sweden) These are based on a tuned oscillator system which becomes off-tuned when a mass is introduced into its lectro-

magnetl field (for det iled description see Svensson and Thieme 1969) I later experiments the motor activity was measured by means of M/P 40 Fc Electronic Motility Meters (Motron Products Sweden) These are based on 40 photoconduc tiv sensors arranged I 5 rows of 8 cell with center to center distance of 40 mm The cell are covered by translucent plastic box The light source is a regular incandescent lamp mounted 115 cm over the photoconducti e sensors I li experiments the motor acti ity of one group of three ani was measured t a tim The recordings were tarted 30 sec after inserting the animal I the test cages The motor activity was I one experiment ecorded conti uously for 6 h and registered as counts from the activity meters per 10 mi (paper III) In other experiments the motor activity was recorded during 20 min and egi tered as ounts per 20 mi The animal were housed under normal lighting conditions The measurements of motor activity were generally performed between 10 00 a m and 3 00 p m

Spinal transections

The descending 5-HT contai ing eurons hav thei cell bodies i the brai stem A transection of the spinal cord will therefor block the flow of nerv impulses and the supply of tryptophan hydroxylase to the nerv terminals below the transection (see Introduction) Rats were spinalized at the midthoraci lev l under ether la Car was taken to mai tai thei normal body temperatur by changing the envi onmental conditions. The urinary bladders of these spinal rats wer emptied twice daily by gentl pressure on the abdomen Unoperated rats serving as control wer given ther anaesthesi unless otherwise tated

Biochemical determinations of monoamines thei precursors and catabolites

Tissues wer homogenized in 10 ml 0 4 N perchloric acid contai ing 20 mg disodium thylene diamine tetraacetate and either 2 mg ascorbi acid or 5 mg $N_2S_2O_5$. The compounds were isol ted from the extracts on columns of cation exchange esi and assayed spectrophotofluorimetrically

In earlier experiments NA and DA wer isol ted on strongly acidi cation exchange column (Dowex 50W X-4) (Bertler Carlsson and Rosengren 1958 as modified by Carlsson and Lindqvist 1962) and 5-HT was isolated on weakly

acidic cation exchange column (Amberlit CG50 type 1) (Bertler 1961 as modified by Andén and Magnusson 1967), and assays were made for NA (Bertler t al 1958) DA (Carlsson and Waldeck 1958 as modified by Carlsson and Lindqvist 1962) and 5-HT (Andén and Magnusson 1967)

In l ter experiments all compounds were isol ted on a single column of the Dowex 50W esi (Atack and Magnusson 1970 Lindqvi t 1971, Kehr Carlsson and Lindqvist 1972) and assays wer mad for tryptophan (Bédard t al 1972) 5-HTP 5-HT and 5-HIAA generally using nati e fluorescenc (see Atack and Lindqvi t 1973) tyrosine (Waalkes and Udenfriend 1957) DOPA (Keh t al 1972) DA (Atack 1973) and NA (Bertler t al 1958)

Model used for the estimation of rate of synthesi of transmitter and of impulse activity in central monoami e neurons

Recently a m thod has been dev loped for determining imultaneously the rate of hydroxyl tion of tryptophan and of tyrosine i ivo (Carlsson Davi Keh Lindqvist and Atack 1972b) Under normal onditions thes hydroxylations are considered to be rate-limiting i the synthesis of 5-HT and CA respecti ely (see Introduction) The products of these hydroxylations 5-HTP and DOPA espectively are normally rapidly decarboxylated and are not readily detectable i the brai After blockade of DC i the CNS the concentr tions of 5-HTP and DOPA increase linearly These accum l tions of 5-HTP and DOPA appear to provide reliabl measures f the rates of synthesi of 5-HT and CA. The concentrations of 5-HTP and DOPA i CNS were determined 30 mi after the administration of NSD 1015 100 mg/kg Thi drug inhibits both DC and MAO and is largely without effect on gross behaviour in animal (see Carlsson t al 1972b) Variations i the rates of synthesis of 5-HT and CA were estimated using ither the abov method (papers VI and VII) or i earlier experiments (papers III and V) by determining estimated accumulations of 5-HT DA and NA after inhibition of MAO (Neff and T zer 1968, Neff and Cost 1968)

Impulse activity i the different monoaminergi neurons i the CNS i considered to be reflected by the rates of depl tion of 5-HT DA and NA after compl te inhibition of thei biosynthesis. Thi i w is supported by the findings that electrical timul tion of NA- or 5-HT contai ing ell bodies accelerates the depl tion of NA and 5-HT respectively whereas the depl tion of 5-HT DA

and NA distal t lesion of the respecti e neurons is almost completely antagonized (Andén Fuxe and Hökfelt 1966; Andén Corrodi and Fuxe 1969b; Andén Corrodi Fuxe and Ungerstedt 1971; Korf Aghajanian and Roth 1973; see also paper V) Furthermore there is good agreement between the effects of drugs on the rate of depl tion of 5-HT after synthesi inhibition and thei effects on the impulse activity i 5-HT neurons as determined from direct recordings i the raphe (compare e g Andén Corrodi Fuxe and Hökfelt 1968; Corrodi and Fuxe 1968 with Aghajanian Foote and Sheard 1970; Sheard Zolovick and Aghajanian 1972) In the present tudy the rate of depletion of 5-HT i brain was determined after the admi i tration of α-propyldopacetamide (H22/54) 500 mg/kg an inhibitor of both tryptophan- and tyrosi e hydroxylase (Carlsson Corrodi and Waldeck 1963)- and the rates of depl tion of DA and NA were measured after the admi istration of DL-α-methyl-p-tyrosine methylester HCl (α-MT) 250 mg/kg which is a f irly specifi inhibitor of tyrosine hydroxylase (Spector Sjoerdsma and Udenfriend 1965; Svensson and Waldeck 1970)

STUDIES ON THE ROLE OF 5-HT IN THE BEHAVIOURAL EFFECTS OF ANTIDEPRESSANT DRUGS

In the treatment of mental depression drugs inhibiting MAO as well as tricycli drugs of the imipram type are well established to be effecti L Tryptophan and 5-HTP the precursors of 5-HT ar eported by some investigators to hav antidepressant properties whereas others have found them to be ineffecti e i this espect The elev tion of mood induced by these various groups of drugs has been proposed to esult largely f om thei common ability to facilitate neurotransmission in entral 5-HT neurons: MAO inhibitors by blocking the catabolism of 5-HT the tricycli drugs by blocking the membrane pump in 5-HT neurons and the precursors by increasing the formation of 5-HT (for eview see g Coppen 1973- Murphy Baker Kotin and Bunney Jr 1973, Brodi Sack and Siever 1973) The effects of these arious groups of drugs on the 5-HT eceptors cannot however be predicted with any certainty i iew of the complex egul tory mechanisms which seem to govern neurotransmission i 5-HT neurons (see introduction)

Ther ar no sensiti models availabl for estimating variations in activity of 5-HT eceptors In consequence the effectiveness of the various groups of antidepressant drugs on the 5-HT eceptors is poorly documented Evidence that the groups of drugs mentioned abov do acti ate 5-HT eceptors is limited to treatments whi h esult i markedly-increased concentrations of 5-HT i the CNS i complet inhibition of MAO and the combined treatment of MAO inhibitors with precursors of 5-HT (see g Corrodi 1966; Meek Fuxe and Andén 1970) When using such treatments the additional admini tration of tri-cycli antidepressant drugs esults i further inc ease i the activation of the 5-HT receptors (Carlsson Jonason Lindqvi t and Fuxe 1969c- Meek t al 1970)

The various groups of antidepressant drugs differ i thei ffects on the behaviour of labor tory animal (vide infra) Attempts wer made i th present investigation to determine to what extent these differences i effect on behaviour reflect variations i activity of entral 5-HT receptors ther by larifying the effectiveness of the various drugs on the 5-HT eceptors. Mi wer used i all experiments and the influence of the various drug treatments on thei motor activity was recorded

Experiments with 5-HTP (papers I and II)

Exogenous 5-HTP is apidly decarboxyl ted to 5-HT both in peripheral and in central tissues which contain DC (Udenfriend W issbach and Bogdanski 1957) The pharmacological ffects of 5-HTP ar generally considered to be medi ted lo the 5-HT so formed (see g Corne Pickering and Warner 1963) The development of DC inhibitors e g MK 486 which poorly penetrate the blood-brai barrier and thereby i hibit peripher l but ot central DC (Porter Watson Titus T taro and Byer 1962; Bartholini and Pl tscher 1969) has made it possibl to block the formation of 5-HT from 5-HTP selecti ly i peripheral tissues When gi en alone 5-HTP i reported to induce sedation or behavioural depression i mice and rats (for review see Mantegazzani 1966) Only few quantitativ tudies are reported of motor acti ity in mice treated with 5-HTP Using photocell counter technique Brown (1960) eported reduced motility i mi e treated with DL 5-HTP i doses from 0 1 to 100 mg/kg whil t Smith and Dews (1962) found no changes i mi treated with DL 5-HTP i doses between 10 and 1000 mg/kg

DL 5-HTP in doses between 6 25 and 50 mg/kg had no ignificant effect on motor activity ither when given lone or i combination with MK-486 Higher doses of DL 5-HTP (100-800 mg/kg) caused reduction i motor activity when alone but dose-dependent increase when given to animal pretreated with 486 The increased motor activity was accompanied by rapid head movements i animal given the highest doses of 5-HTP (400-800 mg/kg) Some of these animal also displayed tremor i thei forelegs and abduction and hyperextension of thei hind legs N igns f aggressiveness or increased irritability were observed The results indicate that the decrease in motor activity induced by large doses of DL 5-HTP is mai ly due to an effect outside the blood-brai barrier since the effect is abolished by inhibition of peripheral DC 5-HTP lso appears to have central effects which induce an i creased motor acti ity these central effects of 5-HTP become obvious only when the peripher l effects of 5-HTP are blocked

When administered i high doses 5-HTP is taken up and decarboxylated not only in 5-HT neurons but also in CA neurons i the brai The 5-HT so formed i the CA neurons displaces thei tores of transmitters (Fux Butcher and Engel 1971) Whether this displacement of CA induced by 5-HTP has any functional significance whereby the eleased CA cause an increased timul tion of central

CA receptors had not been established Therefore larification was sought as to whether the stimulant effect of 5-HTP on motor activity obtained after inhibition of peripheral DC is aused predominantly by an xagger ted effect on central 5-HT eceptors or by indi ect stimulation of central CA eceptors

If the CA neurons were to be pre-emptied of CA prior to admini tration of the 5-HTP (plus MK 486) then per se no CA would be av ilabl for release by 5-HT formed from 5-HTP and no stimulation of CA eceptors could occur T eatment with DL-α-methyl metatyrosi e (α-MMT) is able to induc a depletion of the tores of CA in CA neurons and causes little sedative eff ts (Andén 1964, Chan and Webster 1971) It was gi en to mice i successi injections of 400 400 and 200 mg/kg 27 15 and 2 5 h respecti ly befor the administration of DL 5-HTP

α-MMT gi en alone induced a marked depl tion of DA and NA i brai but had no significant effect on motor acti ity The increase i motor activity induced by DL 5-HTP (plus MK-486) was not antagonized by pr treatment with α-MMT Thus the fact that 5-HTP (plus MK 486) induced similar timulatory effect i animal which were already depleted of thei tores of CA indicates that the d's-placement of CA i unlikely to be of importance for thi action (see also Rubenson 1971) However the possibility emains that 5-HTP causes the elease of small amounts of CA f om pool which are not depl ted by α-MMT

The antidepressant drugs chlorimipramine and pr triptyline can selecti ly block the membrane pump for e-uptak of amines i to 5-HT neurons and NA neurons respecti ely (Carlsson Corrodi Fuxe and Hökf lt 1969a b) Consequently chlorimipramine would potentiate ffects induced by an increased release of 5-HT since its activ e-uptak i to 5-HT neurons i blocked whereas protriptyline would potenti te effects induced by an increased release of NA ince its activ re-uptak i to NA neurons i blocked

Pretreatment with chlorimipramine markedly potentiated the timulatory effect of 5-HTP (plus MK 486) on motor acti ity i mice whereas pretreatment with protriptyline had no such potentiating effect In animal pretreated with chlorimipramine (plus MK 486) DL 5-HTP i doses as low as 12.5 mg/kg induced significant increase i the motor acti ity and maximal increase was obtained after 75 mg/kg, doses higher than 75 mg/kg induced pronounced muscular disturbances Administration of 5-HTP 75 mg/kg to mice pr treated with MK 486 caused no significant lowering of CA oncentrat ons i brai

Th fact that chlorimipramin but not protriptylin potentiated the timulatory effect of 5-HTP on motor activity strongly suggests that this action of chlorimipramine is mediated la its effect on neurotransmission i 5-HT eurons. Thus the increase i motor activity induced by 5-HTP (plus MK 486) would appear to be largely dependent on an increased activation of central 5-HT receptors

An indirect effect of 5-HTP on CA receptors medi ted i displacement of CA from central CA neurons i likely to make little if any contribution to the stimul tory effect of 5-HTP on motor activity since:

1 Pr treatment with α-MMT to empty the CA neurons of thei amine content did not antagoni e the stimulatory effect of 5-HTP (plus MK 486)

2. Motor activity i mic which wer pretreated with chlorimipramine to block amine e-uptak i to 5-HT neurons was markedly increased by a dose of 5-HTP (75 mg/kg after MK 486) producing no igni-ficant lowering of the concentrations of CA in brai

3. If the stimulatory effect induced by DL 5-HTP was largely dependent on an increased release of NA pretreatment with protriptyline to block amine re-uptake into NA neurons should hav potentiated the timul tory effect but no such potentiation was observed

4 DL 5-HTP i dose of 12.5 mg/kg given to mice pretreated with chlorimipramine (and MK 486) induced a significant increase i motor activity, whereas dose of 250 mg/kg of L DOPA the corresponding precursor i the synthesis of CA was found by trömberg (1970) to be required to induce an increase i motor activity i mi e pretreated with MK 486 Therefore if the increase in motor activity induced by DL 5-HTP is medi ted mai ly i an increased acti tion of CA receptors one must assume that DL 5-HTP is far more effective i this respect that equimolar doses of L DOPA.

5 In addition it is noteworthy that no signs f aggressiveness or increased irritability were observed during th experiments These signs often accompany timulation induced by drugs which activat CA eceptors (vide infra)

Experiments with nialamide (paper III)

Inhibition of MAO results i the accumul tion of 5-HT and CA in monoamine neurons and overflow of these monoamines from nerv terminals (Carlsson Dahl tröm Fuxe and Lindqvist 1965) Large doses of MAO inhibitors hav a psychomotor timulant effect These biochemical and behaviour l effects seem to be causally related (Carlsson et al 1963 Carlsson t l 1965 for evi w see Pletscher 1966)

The behavioural syndrome induced in mi by inhibiting MAO with ialamide was reported by Corrodi (1966) to be antagonized by pretreatment with drugs which block the accumulation of both 5-HT and CA (i e DL-α-propyldopac tamide and L-α-methyldopa) but not by pretreatment with a drug which selectively blocks the accumulation of CA (α-MT) Consequently the accumul tion of 5-HT i the brain was assumed to be of predominant importanc for the nialamide-induced timulation of psychomotor acti ity Thi iew i also supported by findings that the 5-HT precursors L tryptophan and 5-HTP enhance the behavioural effects of MAO inhibition (Grahame-Smith 1971; for revi w see Mantegazzani 1962) The availability of p-chlorophenylalanine a fairly specific inhibitor of tryptophan hydroxylase (Koe and Weissman 1966, Jéquier Lovenberg and Sjoerdsma 1967) prompted further evaluation of the respectiv roles of accumul ted 5-HT and CA i the timulant ffect of nialamide on psychomotor activity

Nialamide HCl (200 mg/kg) li ited an initial depression of motor activity i mice followed by an increase in activity which was maximal approximately 4 h after the nialamide i jection The increase i motor acti ity was i itially accompanied by hypertherm and rapid head movements, and subsequently also by tremor and by hyperextension and abduction of the hind legs. These motor di turbances become pronounced after 4 h with an associ ted decline i the egistered motor activity Nialamide induced more pronounced accumulation of 5-HT than of DA or NA in brai when measured 1 3 and 6 h after its i jection P treatment with L tryptophan 300 mg/kg 1 h before giving ialamid esulted i a more rapid onset of the psychomotor timul tion and faster accumul tion of 5-HT than was obtained after ialamide alone These behavioural and biochemical effects of L tryptophan are i all probability causally l ted (cf Grahame-Smith 1971)

PCPA admini tered alone to mi i ither of the two dose egimens mentioned

below was largely without effect on motor acti ity It induced a marked decrease i the concentration of 5-HT but not of CA i brai Pretreatment with PCPA 400 mg/kg 24 h before giving nialamid partially antagonized th increase i motor acti ity and accumulation of 5-HT in brai which are both induced by ialamide alone Pretreatment with PCPA 800 mg/kg for 3 consecutive days at 72 48 and 24 h before giving i lamide completely antagoni ed the behavioural excitation and hypertherm i and the accumul tion of 5-HT which are induced by ialamid lone However thi l tter pretreatment with PCPA also partially antagoni ed the ni lamide-induced accumulation of DA and NA in brain indicating an impaired synthesis of CA i these animal (Tagliamonte Tagliamonte Corsi i Mereu and Gessa 1973)

The additional admi i tration of DL 5-HTP 30 mg/kg 1 h after giving ialamide to the animal thrice pretreated with PCPA restored th behaviour l excitation The results indicat that the antagoni ing effect of PCPA on the ialamide syndrome is largely due to its inhibitory ffect on the synthesi of 5-HT The inhibitory ffect f the tripl dose f PCPA on the synthesi of CA may possibly contribut to th compl t antagonism of th syndrom However the finding that relatively low dose of 5-HTP compl tely restored the i lamide-induced syndrom i animal thrice pretreated with PCPA suggests that the PCPA-induced i hibition of CA synthesi i not of great importanc

α-MT admi i tered lone to inhibit tyrosine hydroxylase lowered the concentrations of DA and NA i brai Pr treatment with α-MT 200 mg/kg 2 h before ialamide reduced th i lamide-induced hyperactivity to about the same extent as did pretreatment with PCPA 400 mg/kg and ompl tely antagonized the ialamide-induced hypertherm i Also th accumulation of CA which i induced by i lamide lone was ompletely blocked by α-MT indicating an ffecti inhibition of CA synthesis The contribution f entral CA eurons to th maintenance of normal motor acti ity i fairly well established (see g Carlsson Lindqvi t and Magnusson 1957; an Rossum 1970; Svensson 1971; Ahlenius Andén and Engel 1973) and blockade of the CA synthesis esults in depression of motor acti ity (see g Svensson and Waldeck 1970) The partial inhibition of the ni lamide-induced hyperactivity i animal pr treated with α-MT indicates that the fully-dev loped ialamide yndrome i t least dependent on fun tionally-i tact central CA neurons. The finding that i lamid nevertheless i creased the

motor acti ity in animals pretreated with α-MT emphasizes the rol of 5-HT in psychomotor stimulation

The initial depression of motor acti ity observed after the admi istration of nialamide was not antagonized by pretreatment with either PCPA or α-MT Consequently this behavioural effect seems to be unrel ted to the changes in 5-HT and CA concentrations i brain.

In conclusion the hyperactivity induced by nialamide seems to be largely dependent on the inhibition of 5-HT catabolism and hence like the qualitatively similar 5-HTP induced hyperactivity effects an increased activ tion of central 5-HT eceptors. Possibly an increased activation of CA receptors may contribute to some extent At least the fully developed hyperacti ity seems to be dependent on an intact neurotransmission i entral CA neurons

Experiments with L tryptophan (paper IV)

Tryptophan plus metaboli inhibitors

L Tryptophan i metabolized along several pathways both in peripheral tissues and in the CNS (for eview see e g Guroff and Lovenberg 1970) These include the formation of 5-HT (see Introduction) and of tryptamine (Werle and Mennicken 1937; Hess Redfield and Udenfriend 1959; Weissbach King Sjoerdsma and Udenfriend 1959) A quantitati ely important route i the oxidation of tryptophan in liver tryptophan pyrrolase to formylkynurenine in the "kynurenine-pathway (Greengard and Feigelson 1962; Nemeth 1962)

L Tryptophan induces an increase i the synthesis of 5-HT i brai which differs from that induced by 5-HTP in two ways: 1) The maximal increase in 5-HT synthesis which can be obtained by admi istration of L tryptophan i much less pronounced than that which can be obtained by administration of 5-HTP and the tryptophan hydroxylase becomes saturated by lower doses of its substrate than does the DC. The maximal synthesi of 5-HT which could be obtained i mouse brain after admini tration of L tryptophan equired 300 mg/kg when given intraperitoneally (Carlsson and Lindqvi t 1972) 2) The 5-HT formed i the brai from exogenous L-tryptophan appears to be locali ed selecti ely i the 5-HT neurons as result of the specifi distribution of tryptophan hydroxylase (see Introduction), whereas when 5-HTP i admini tered the 5-HT so formed i less specifically localized

(Id supra) L Tryptophan I eported to reduce the spontaneous motor activity I l boratory animal (Brown 1960; Ashcroft Eccleston and Crawford 1965) and to induc sedation and changes of the sleep-pattern i man (see Wyatt Engelman Kupfer From Sjoerdsma and Snyder 1970; Hartman Chung and Chien 1971) These two latter effects of L tryptophan have also been observed in patients pre-treated with p-chlorophenylalanine and have consequently been suggested to be unrel ted to the increase i 5-HT synthesis induced by L tryptophan Further clarification of the centr l effects of tryptophan on motor activity was therefore sought using inhibitors of the different metabolic pathways for L tryptophan

L Tryptophan 50-800 mg/kg was admi istered to mic and their motor acti ity was recorded 1 h later Groups of animal were pretreated with drugs which inhibit the above-mentioned metabolic pathways of L tryptophan Pretreatment with PCPA (800 mg/kg on three consecutiv days) was used to inhibit th tryptophan hydroxylase MK 486 (75 mg/kg given 30 mi before tryptophan) was used to selectively inhibit peripheral DC and NSD 1015 (100 mg/kg given 30 mi before tryptophan) was used to inhibit both entral and peripheral DC, thus i hibiting the formation of monoamines i cluding tryptamine Allopurinol (50 mg/kg given 2.5 h befor tryptophan) was used as an inhibitor of tryptophan pyrrolase (Becking and Johnson 1967)

Animal given 800 mg/kg of L tryptophan appeared sedated and thei motor acti ity was significantly reduced Lower doses of L tryptophan had no apparent effects on gross behaviour and did not significantly affect the motor acti ity None of the various pretreatments antagoni ed the L tryptophan induced reduction i motor activity: pretreatment with PCPA was ineffecti whereas pr treatment with MK-486 NSD 1015 or allopurinol potenti ted the behavioural depressant effect of L tryptophan i e the dose-response curv was shifted to th left i these latter groups of animal The results indicate that the L tryptophan induced reduction i motor acti ity is not mainly mediated i any f its known neurotropi metabolites i e 5-HT tryptami or metabolites along the kynurenine pathway Possibly therefore the behavioural-depressant effect of L tryptophan may be mediated by the amino acid itself ther than i any of its metabolites. In iew of the sedative effect of L tryptophan i man it seems likely that its inhibitory effect on motor activity i mice i at least parti lly of central origi

Tryptophan plus chlorimipramine:

Chlorimipramine 25 mg/kg was gi en to mice 30 mi before admini tration of L tryptophan 50-800 mg/kg When compared with animal given only chlorimipramine (plus saline) mice given 100 mg/kg L tryptophan exhibited a moderate increase i motor acti ity whereas after 800 mg/kg motor acti ty was reduced and doses of 50 200 and 400 mg/kg were ineffective

The hyperacti ity induced both by 5-HTP and by lolamide i medi ted by 5-HT and is potentiated when re-uptak of transmitter into 5-HT neurons is blocked by chlorimipramine (vide supr) Consequently the administration of 100 mg/kg L tryptophan may well induce an increased elease of 5-HT which in the chlorimipramine pretreated animal may be suffi ient to cause an increased acti tion of central 5-HT receptors; this being reflected as an increase i motor activity

Clinical and physiological implications

In the experiments elating to the administration of DL 5-HTP or lolamide to mice a gradual increase in motor acti ity appeared to be correlated with a gradually increased release of 5-HT f om entral 5-HT neurons Henc in these experiments the hyperacti ity seems to provide quantitative measure of the activation of 5-HT receptors Hyperacti ity appeared to result from a marked over timulation of 5-HT receptors However the absence of hyperactivity i animals treated with either L tryptophan or the lower doses of DL-5-HTP with or without pretreatment with MK-486 does not exclude the possibility that the increase obtained in the synthesis of 5-HT after these treatments may have resulted in moderately increased release of 5-HT and activation of 5-HT eceptors. The experiments with DL 5-HTP illustrate this: dose of DL-5-HTP as low as 12.5 mg/kg increased the motor acti ity i animal pretreated with chlorimipramine plus MK-486 whereas 100 mg/kg was the lowest effective dose of DL 5-HTP in animal pretreated with MK 486 alone The potentiation is i all probability mainly due to the membrane pump blocking effect of chlorimipramine esulting in an enhanced concentration of 5-HT at the postsynapti receptors. Thus the results indicate that also when DL-5-HTP 12.5 mg/kg is administered to animals pretreated with MK-486 alone t least some increase in the elease of 5-HT i probably induced

The increase i motor acti ity induced by L tryptophan 100 mg/kg after pretreatment with chlorimipramine suggests for the same reason that this dose of L tryptophan induces an increased release of 5-HT also when the drug is given lone

A comparison between the two percursors of 5-HT reveals that DL 5-HTP gi en after inhibition of peripheral DC has much higher efficacy than L tryptophan with regard to activ tion of central 5-HT eceptors Admi istr tion of high doses of DL 5-HTP after pretreatment with MK 486 esults i pronounced overstimul tion of 5-HT receptors This overstimulation is comparabl to that obtained after compl te inhibition of MAO. Presumably the inhibition of peripheral DC contributes considerably to the high efficacy of DL 5-HTP (see g Horita and Hamilton 1970 and i troduction) Evidence for the increased acti ation of central 5-HT receptors afte admi i tration of L tryptophan alone has not been presented However thi lack of evidence may merely reflect the unavailability of sensiti model for estimating the acti ity of 5-HT receptors The moderate stimulant effect on behaviour which L tryptophan 100 mg/kg was found to induce i chlorimi pramine-pretreated animal i suggestive evidence for the activation of 5-HT receptors by the 5-HT formed from the admi istered amino acid Although thi effect is reproducibl the finding equi es further study before a defi ite conclusion can be

The 5-HT formed i CA neurons after admi i tration of high doses of DL 5-HTP did not seem to contribute to the behavioural effects of the precursor DL 5-HTP would therefor appear to exert its functional effects fairly specifically i 5-HT neurons. In contrast high doses of L tryptophan were found to induce behavioural depression by a mechanism unrel ted to 5-HT Furthermore high doses of L tryptophan hav neuroleptic-lik effect on conditioned avoidance behaviour i rats (Engel and Modigh 1974) and induce a partial inhibition of the synthesi of CA i mouse brain (Modigh unpublished observation) These unspecifi effects of L tryptophan are only obtained after gi ing doses which are i excess of that required to cause saturation of br i tryptophan hydroxylase For more accurately interpreting the antidepressant ffect of L tryptophan there would thus appear to be an urgent need to determine the dose level at which the brai tryptophan hydroxylase i man is saturated Tryptophan has been cl imed to be a considerably more specific tool with respect to central 5-HT neurons than 5-HTP (see g. Moi and Eccleston 1968) The present results do not support this assumption

The ability of antidepressant drugs to elevate mood is widely assumed to esult from a facilitation of neurotransmission i 5-HT neurons. Clinical evaluations of the effects of 5-HTP i the treatment of endogenous depressions are rar and contradi tory (vide supra) but i i w of the present findings further tudy appears to be warranted. DL 5-HTP admini tered i combination with an inhibitor of peripheral DC alone or together with chlorimipramine seems to acti t 5-HT receptors fairly specifically; moreover such treatments ar equally as ffective as the use of MAO inhibitors Overdosages of these treatments esult i serious intoxications mainly due to overstimul tion of 5-HT eceptors (vide inf) However the effects of 5-HTP are of shorter duration than those of MAO inhibitors Therefore the use of 5-HTP in combination with an inhibitor of peripheral DC alone or together with chlorimipramine would appear to be safer than the use of MAO inhibitors when the therapeuti aim is to achieve an effecti e activation of entral 5-HT eceptors

Overdosage of MAO inhibitors whether given alone or i combination with tricyclic antidepressant drugs esults i intoxications which are sometimes lethal (see g Simmons Carr and Ros 1970; Matthew 1973) The present investigations indicate that 5-HT plays predominant rol i such i toxications as for exampl supported by the finding that PCPA blocked both the behavioural xcitation and the mortality esulting from compl te inhibition of MAO S mi Henc the admini tration of drugs which inhibit the synthesi of 5-HT should provid causal therapy for such intoxications

5-HT i the CNS was originally proposed on the basi of esults of experiments with eserpine to serv as modul tor of a trophotropi system which when activated would cause behavioural depression and sleep (Brodi and Shor 1957; Brodi Comer Costa and Dlabac 1966; Brodi and Reid 1968) Considerabl evidence now indicates that the sedation induced by reserpine i due mainly to an impaired neurotransmission in central CA neurons and is largely unrel ted to effects on the 5-HT neurons (see g Carlsson t al 1957; Carlsson 1965; Butcher Rhodes and Yuwiler 1972) The esults of mor ecent tudies of behavioural effects i ted to an impaired neurotransmission in 5-HT neurons ar i agreement with the original hypothesi proposed by Brodi and co-workers. pharmacological blockade of the 5-HT neurons leads to an increased reactivity to external timuli (see g Koe and Weissman 1966; Brody Jr 1970; Butcher and Di trich 1973) and to

Insomni (for review see Jouvet 1973) Moreover lesion of raphe neurons esults I behavioural excitation (Kostowski Giacolone Garattini and Valzelli 1968) and insomnia (see Jouv t 1973) However lectrical timulation of raphe cells does not induce sedation (Aghajanian and Sheard 1968) Furthermore I all the present experiments I whi h an increased acti ity of 5-HT eceptors was induced no behavioural depression was observed which could be rel ted to an increased activation of 5-HT eceptors Hence there I evidence I support of the iew that central 5-HT neurons are invol ed in the i tegration of external stimuli and in the egulation of sleep. Howeve 5-HT does not seem to act as an inhibitory modu-l tor In the sense that physiologically increased acti ity I the 5-HT neurons would be expected to result in sedation

STUDIES ON INTERRELATIONS BETWEEN NERVOUS ACTIVITY AND SYNTHESIS OF TRANSMITTER IN 5-HT NEURONS

Experiments with chlorimipramine and PCPA (papers V and VI)

The admini tration of chlorimipramine or rel ted inhibitors of the membrane pump of 5-HT neurons suppresses the impulse acti ity in 5-HT neurons (Sheard et al 1972; see also Carrodi and Fuxe 1968) and partially inhibits the synthesis of 5-HT (Meek and Werdinius 1970 Schubert Nyback and Sedvall 1970; Schildkraut Schonberg Breese and Kopin 1969; Bruinvel 1972) The suppression of impulse activity seems likely to be a feed-back phenomenon which i elicited by the increased activation of pre- or postsynaptic 5-HT eceptors following the inhibition of amine re-uptake (vide supra) The acti ity of brai tryptophan hydroxylase appears from lesion and stimulation experiments to be partially dependent on the impulse acti ity of 5-HT neurons (see Introduction) Hence the inhibitory effect of chlorimipramine and related drugs on the synthesis of 5-HT may be secondary to thei inhibitory effect on the impulse activity as has been suggested by Schubert et al (1970) Alternatively these drugs may directly inhibit the tryptophan hydroxylase or the uptake of tryptophan into the 5-HT neurons Thi latter possibility has been emphasized by Bruinvel (1972) who found that imipramine competitively inhibits the uptak of L tryptophan i to synaptosomes.

A possible relation betwen inhibition of the nerve impulse acti ity and inhibition of the 5-HTsynthesis after administration of chlorimipramine was investigated by studying the effects of the drug on the rate of tryptophan hydroxylation in the rat spinal cord above and below thoraci transection (see Methodology) For comparison the effects of PCPA which directly inhibits tryptophan hydroxylase (vide supra) were tudied in parallel The rate of synthesis of 5-HT was estimated i one of two ways: 1) I the experiments using chlorimipramine the accumulation of 5-HTP 30 mi after administration of NSD 1015 100 mg/kg referred to as 5-HTP (after NSD 1015) was measured 2) when using PCPA the accumul tion of 5-HT 2 1/2 and 5 h after administration of nialamide HCl 500 mg/kg referred to as: 5-HT (after ialamide) was measured After an acute spinal transection the accumul tion of 5-HTP (after NSD 1015) was educed by approximately 40 per cent i the spinal cord bel w the transection but was not significantly effected i the cranial part By contrast the acute transection did not signifi-

cantly affect the accumulation of 5-HT (after nialamide) in either of the two hal es of the spinal ord These results are in agreement with earlier eports (Carlsson et al 1973b Meek and Fuxe 1971) The educed accumul tion of 5-HTP (after NSD 1015) below a spinal transection indicates that the synthesis of 5-HT was decreased in the caudal part probably due to blockade of the nerve impulse flow

Administration of MAO inhibitors soon leads to a partial inhibition of the 5-HT synthesis (Macon t al 1971; Carlsson Bédard Davi Kehr Lindqvist and Magnusson 1973a) and to an almost complete inhibition of the impulse acti ity i the 5-HT neurons (Aghajanian Graham and Sheard 1970) The difference in impulse activity in 5-HT neurons abov and below spinal transection i therefore largely abolished shortly after the admi istration of nialamide Thus the finding that the accumulation of 5-HT (after ialamide) is unaffected by spinal transection supports the iew that the reduced accumulation of 5-HTP (after NSD 1015) below an acute spinal transection i due to blockade of the impulse flow i the 5-HT neurons and not to other consequences of the transection

When PCPA 400 mg/kg was admini tered to rats 1 h after spinal transection then 24 h l ter the 5-HT ynthesi (5-HT accumulation after nialamid) was found to be equally inhibited both abov and below the transection

Chlorimipramine 15 mg/kg admi istered 2 h after a spinal transection ced the 5-HT synthesis (5-HTP accumul tion after NSD 1015) i the brain and the ranial part of the spinal cord; but below the transection the 5-HT ynthesi was not ignificantly affected After the chlorimipramine the concentration of tryptophan i the CNS was not hanged i either intact or transected animal

The different effects of PCPA and chlorimipramine on the rate f 5-HT ynthesi in the spinal cord below transection indicate that the two drugs must inhibit the 5-HT synthesis by different mechanisms The ineffecti eness of chlorimipramine below spinal transection i i keeping with the iew that the drug inhibits the 5-HT ynthesis ia depression of the nerv impulse acti ity

Reserpine 10 mg/kg given 4 h before chlorimipramine 15 mg/kg was found to prevent the inhibitory effect of chlorimipramine on the accumulation of 5-HTP (after NSD 1015) (table I)- reserpine itself i reported not to ignificantly affect thi accumul tion of 5-HTP (Carlsson and Lindqvist 1972; compare also the control groups i tabl I and fig I) In contrast PCPA given to reserpine-pretreated animal effectively inhibits the 5-HT synthesi (5-HT accumulation after nialamide)

Table I. Concentrations of 5-HTP and tryptophan in rat brain 30 min after the administration of NSD 1015 100 mg/kg The animal were pr treated with reserpine 10 mg/kg t 4 1/2 h and either chlorimipramine 15 mg/kg or 0 9% w/ NaCl at 30 min befor the admini tration of NSD 1015 The drugs were administered by the I traperitoneal route Each determination of 5-HTP and tryptophan was made from singl whole brain Each alue represents the mean of 7 determinations ± s. m

Pretreatment before NSD 1015	5-HTP µg/g	Tryptophan µg/g
Reserpine + 0 9% saline	0 097 ± 0 007	5.69 ± 0 08
Reserpine + chlorimipramine	0 110 ± 0 012	5 63 ± 0.09

(Koe and Weissman 1966) The mai effect of reserpine is to block the function of the storage granules I monoamine neurons (for review see Carlsson 1965) which in turn nullifies the ability of the nerve impulses to release the monoaminergi transmitters Therefore after pretreatment with reserpi e the blockade of th membrane pump induced by chlorimipramine may be assumed to be functionally ineffectiv at the 5-HT receptors Furthermore reserpi is reported to partially depress the impulse activity i 5-HT neurons (Aghajanian and Haigler 1973) Consequently the antagoni ing effect of reserpine on the inhibition of the 5-HT synthesis induced by chlorimipramine i in agreement with the feed-back hypothesi mentioned above

Chlorimipramine given in various doses (3 75-60 mg/kg) was studied for its effects on the rate of synthesi of 5-HT (accumulation of 5-HTP after NSD 1015) in brai and i crani l and caudal spinal cord of unoper ted rats (fig 1) Each dose of chlorimipramine gave imilar effect i the three parts of the CNS. 3.75 mg/kg of chlorimipramine had no ignifi ant ffect whereas doses between 7 5 and 60 mg/kg wer lmost equally effective in reducing the accumulation of 5-HTP by approximately 35 per cent Thi reduction i of the same magnitude as that obtained i the caudal part of the spinal cord after an acute midthoraci transection (vide supra) Interestingly the smallest effective dose i the present experiments is almost identical to the ED 50 (i traperitoneal admi i tration in mice)

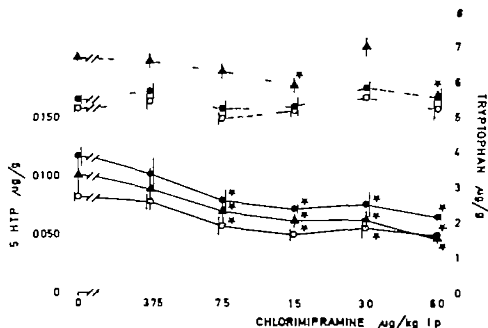


Fig 1 Eff ts of arious doses of chlorimipramine given 30 mi before NSD 1015 100 mg/kg on the con entration of 5-HTP (———) and tryptophan (-) i the brai (▲) and i the cranial (O) and caudal parts (●) of the spinal cord of unoperated unanaesthetized rats The drugs were admi istered by the i traperitoneal route The animal were killed 30 mi after the i ject on f NSD 1015 For *each determination of 5-HTP and tryptophan one whol brai or four* pooled hal es of the spinal cord were used Each poi t represents the mean of 6 determinations ± s m The data were evaluated tatistically by means of analysi f variance followed by t-test (Winer 1962) ★represents p<0 05 i comparison to control group gi en 0 9% saline sol tion plus NSD 1015 (=chlorimipramine 0 mg/kg)

for the chlorimipramine-induced blockade of the membrane pump i 5-HT neurons (Carlsson 1970) If hlorimipramine i hibited the 5-HT synthesi mai ly by competitiv ly inhibiting th uptake of tryptophan i to 5-HT neurons as was suggested for imipramine (ide supra) then the inhibition of the accumulation of 5-HTP would have been expected to be increasingly effective after doses of chlorimipramine higher than 7 5 mg/kg

Th concentration of tryptophan i the spinal cord was not ignificantly hanged by any of the doses of chlorimipramine (Fig 1) The br i concentration

of tryptophan was lightly but significantly reduced by chlorimipramine in the doses 15 and 60 mg/kg whereas the other doses had no significant effects. Some of the animals given the highest dose of chlorimipramine (60 mg/kg) showed toxic reactions. The largely unchanged concentr tions of tryptophan found after chlorimipramine suggests that the drug in the dose range investigated has little if any effect on the uptake of tryptophan into the CNS.

In conclusion the present experiments show that PCPA and chlorimipramine inhibit the synthesis of 5-HT by different mechanisms the inhibition induced by chlorimipramine probably being mediated mainly via inhibition of the impulse flow in 5-HT neurons. Consequently the esults also gi e additional support to the iew that the 5-HT synthesis is to some tent regulated by the nerve mpulse activity.

PCPA

PCPA inhibits the brai tryptophan hydroxylase both i vo and i vitro. The inhibition of the enzyme in ivo becomes irreversible after an initially reversible phase (Koe and W isman 1966; Jéquier et al 1967) but the mechanism of the irreversibl inhibition has not been completely clarified (for review see Koe 1971). Gál and co-workers hav shown that PCPA is incorporated i to different enzyme proteins including phenylalanine 4-hydroxylase i liver and proteins with tryptophan hydroxylase activity in brain. They postul ted that the irreversible inhibition of tryptophan hydroxylase by PCPA i due to its incorporation into newly-built enzyme protein at or near to the active ite (Gál Roggeveen and Millard 1970; Gál and Millard 1971). However in the present experiments the inhibition of 5-HT synthesis (accumulation of 5-HT after nialamide) was the same whether PCPA was given to animal spinally transected 1 h earlier or to intact animals. A spinal transection blocks the supply of newly-formed tryptophan hydroxylase to the spinal cord below the transection (see Methodology). Therefore the result indicates that 24 h after the administration of PCPA the inhibit on of 5-HT synthesi is not dependent on the incorporation of PCPA into the newly-formed enzyme. Thi inhibition i probably mainly irreversibl (see Jéquier t al 1967). The results also indicate a slow turnover of tryptophan hydroxylase in the spinal cord which is i agreement with eport by Meek and Neff (1972).

Henc the differen es in the effect of fighting on 5-HT depletion and on 5-HTP accumulation may to som extent reflect possibl ariations in stress I tensity A mor likely explanation for the different effects of fighting on 5-HT depletion and on 5-HTP accumulation i that th ate of 5-HT synthesis increases as result of the increase I concentration of brai tryptophan (Tagliamonte Tagliamonte Per z-Cruet Stern and Gessa 1971; Carlsson and Lindqvist 1972; Curzon Joseph and Knott 1972) whereas the impulse flow I the 5-HT neurons I largely unchanged Therefore the increase in 5-HT metabolism during fighting I unlikely to flect correspondingly I creased acti ation of postsynapti 5-HT eceptors

Effects of isol tion nd fighting on CA i brains

The synthesis and lease of the CA I brai during the isolation and fighting expe iments were tudied i paralI I with the 5-HT synthesi was measured by determi ing the rate f accumul tion of DOPA after admi I tration of NSD 1015 100 mg/kg; and release by determi ing the rate of depletion of DA and NA after admi I tration of α-MT 250 mg/kg (see Methodologi al considerations) In brief DOPA accumul ted at lower rate I "isol ted than I grouped" animals and the accumulation was markedly accelerated during fighting Sinc these differences DOPA accumulation were equally pronounced I DA- and NA rich areas of the IN they probably eflect differences I the rates of ynthesi of both DA and NA he depl tion of DA and NA induced by α-MT was sl wer I isol ted (see W lch and Welch 1968b) than i grouped animal and was accelerated during fighting The results indicate that the impulse fl w and rate of synthesis of trans-mitter I DA-and NA neurons I brai re retarded during isolation but ar rapidly accelerated during fighting The rate of synthesis of CA I generally considered to be regul ted mai ly by the impulse flow I th CA neurons (for review see Andén t al 1969a) Hence the observed differences I CA synthesis are likely to be econdary to the differences i impulse flow Central NA and DA neurons hav both been proposed to be involved I the expression of aggressi behaviour (see g Fog 1969; Randrup and Munkvad 1969; W lch and W lch 1969; McKenzi 1971) The present result supports this iew

Clinical and physiological implications

Variations in the rat of 5-HT synthesis which are elated to changes of the impulse flow i 5-HT neurons appear from experiments using stimulation and lesions to be due to ari tions i acti ity of the tryptophan hydroxylase rather than to variations i substrate concentration near the enzyme (Shields and Ec leston 1972; Carlsson t al 1972). Curzon and co-workers have suggested that the activity in 5-HT neurons may also i fluenc mechanisms whi h determine the concentration of brai tryptophan i e an increased demand for 5-HT would i a feed-back mechanism induce an increased concentr tion of tryptophan i the brain. This hypothesi is based on the finding of statistically significant correlation between the increased concentrations of tryptophan and 5-HIAA in brain during tress (Curzon t al 1972; Knott Joseph and Curzon 1973) Similar biochemical effects were found i the present experiments when studying the tress of fighting However these effects did not seem to be accompanied by an increased impulse flow in the 5-HT neurons Hence at least during the tress of fighting changes in the 5-HT ynthesi may occur which ar unrelated to the impulse activity i 5-HT neurons.

In iew of the above considerations the functional activity i 5-HT neurons is probably more reliably reflected by estimating the tryptophan hydroxylase activity than by estimation of the total turnover of 5-HT At present i clinical tudies of the possible invol ement of 5-HT i mental diseases estimations of the total turnover of 5-HT i the CN are made from measurements of the accumulation of 5-HIAA in samples of cerebrospinal fluid (CSF) after probenecid treatment (see e g Roos and Sjöström 1969; Sjöström and Roos 1972) No li ically-applicabl method for estimating the acti ity of brai tryptophan hydroxylase i man has yet been developed Possibly a ratio between the concentrations of tryptophan and 5-HIAA i the CSF may be an index of the acti ity of this enzyme If such a relationship could be demonstrated experimentally i laboratory animals, then the concomitant determination of 5-HIAA and tryptophan i human CSF should provi a means of estimating brain tryptophan hydroxylase i man This i turn would probably more accurately reflect the functional acti ity i 5-HT neurons.

Hence the differences i the eff ct of fighting on 5-HT depletion and on 5-HTP accumul tion may to som extent reflect possible ari tions i tress i tensity A more lik ly explanation for the different effects of fighting on 5-HT depletion and on 5-HTP accumul tion i that the rate of 5-HT synthesis i creases as a result of the increase i concentration of brai tryptophan (Tagliamont Tagliamonte Perez Cruet Stern and Gessa 1971; Carlsson and Lindqvi t 1972; Curzon Joseph and Knott 1972) whereas th impulse flow i the 5-HT neurons i largely unchanged Therefore the increase i 5-HT metabolism during fighting is unlikely to reflect correspondingly increased activation of postsynapti 5-HT eceptors

Effects of isol tion and fighting on CA i brai

The synthesis and release of th CA i brai during the isol tion and fighting experiments were tudied i parall l with the 5-HT synthesis was measured by determi ing the rate of accumulation of DOPA after admi istration of NSD 1015 100 mg/kg; and release by determi ing the rate of depl tion of DA and NA after admi istration of a-MT 250 mg/kg (see Methodological considerations) In bri f DOPA accumulated t lower te i isolated than i grouped animals and the accumul tion was markedly accelerated during fighting Since these differences n DOPA accumul tion were equally pronounced i DA- and NA ri h areas f the they probably reflect differences i the rates of synthesi of both DA and NA The depl tion of DA and NA induced by a-MT was lowe i isol ted* (see Welch and Welch 1968b) than in grouped animal and was accelerated during fighting The results indicate that the impulse fl w and rate of synthesi of transmitter i DA-and NA neurons i brain are retarded during isolation but are rapidly accelerated during fighting The rate of synthesi f CA is generally considered to be regul ted mai ly by the impulse flow i th CA neurons (for eview see Andén t l 1969a) Henc the observed differences i CA synthesi are lik ly to be secondary to the differen es i impulse flow Central NA and DA neurons hav both been proposed to be invol ed i the expression of aggressive behaviour (see e g Fog 1969; Randrup and Munkvad 1969; Welch and W lch 1969 McKenzie 1971) The present result supports thi iew

the 5-HT formed i 5-HT neurons or to the 5-HT formed i CA neurons. Pre-treatment with DL α-methyl-metatyrosine i a dose which effecti ely depleted the stores of CA in brain did not counteract the stimulant effect of MK 486 plus DL 5-HTP on motor acti ity Pretreatment with chlorimipramine i dose whi h inhibits f l ly specifically the re-uptak of transmitter i to 5-HT neurons markedly potentiated the psychomotor stimulant effect of MK 486 plus DL 5-HTP Protriptyline administered in a dose which inhibits the re-uptak of transmitter i t NA neurons but not i to 5-HT neurons had no such potentiating effect The results indicat that the hyperacti ity induced by MK 486 plus DL 5-HTP is mainly due to an increased formation of 5-HT in and release of th transmitter from 5-HT neurons The 5-HT formed i CA neurons i br in after admi istration of DL 5-HTP does not appear to induce any marked effects on the function of these neurons. In conclusion DL 5-HTP admini tered after inhibition of peripheral DC appears to be as effective as nialamide for inducing timulation of 5-HT receptors i the brai and 5-HTP exerts its functional effects relati ely specifically i the 5-HT neurons.

3 L-tryptophan when admi istered i dose i excess of that equi ed to saturate brai tryptophan hydroxylase was found to reduce the motor activity i mice Doses of L tryptophan which do not cause saturation of the enzyme induced no apparent behavioural effects Pretreatment with drugs inhibiting ither tryptophan hydroxylase (i e PCPA) peripheral DC (i MK 486) or both peripheral and central DC (i e NSD 1015) or pyrrolase (i allopurinol) did not antagoni e the behavioural depression induced by large dose of L tryptophan On the contrary several of these pretreatments potentiated the behavioural depressant effect of L tryptophan The results indicate that the behavioural depression is not primarily mediated by the neurotropic metabolites of L tryptophan i 5-HT tryptamine or metabolites along the kynurenine pathway Animal pr treated with chlorimipramine showed slight but tati tically significant increase i motor activity after admi istering L tryptophan i dose somewhat lower than that equired to saturate brai tryptophan hydroxylase Thi finding i suggestive evi-dence for an increased activation of central 5-HT receptors by 5-HT formed from administered L tryptophan In conclusion the results indicate that administration of L-tryptophan gi es ise to an increased activation of central 5-HT receptors.

L Tryptophan has however a much lower efficacy i this espect than does DL 5-HTP administered after inhibition of peripheral DC. Furthermore high doses of L tryptophan induce behavioural effects by mechanisms unrel ted to central 5-HT neurons.

4 No igns of behavioural depression relating to an increased activation of central 5-HT receptors were found i any of the experiments described abov On the contrary treatment with drugs which increased the timul tion of central 5-HT receptors induced behavioural excitation Thus the present results are i opposition to the concept that 5-HT acts as an inhibitory modulator" i the sense that physiologically-increased activity i the 5-HT neurons would be expected to result i sedation

5. Chlorimipramine has been previously eported to lower the te of synthesis of 5-HT and to almost compl tely suppress the firing i raphe neurons. The suppression of the nerv impulse acti ity i likely t be a feed-back phenomenon eli ited by an increased activation of pre- or postsynapti receptors after inhibition of the re-uptak of transmitter i t the 5-HT neurons. Attempts were made to ify whether the reduction i 5-HT synthesi obtained after admi i tration of iprami i an eff t secondary to the suppression of the impulse activity 5-HT neurons or is due to direct inhibitory effect on 5-HT ynthesi Admi i tr tion of chlorimipramine to rats educed the rate of tryptophan hydroxyl tion which was estimated in brai and spinal cord i ivo by the accumulation of 5-HTP after inhibition of DC A midthoraci transection of the spinal cord blocks the impulse flow i 5-HT neurons below the transection In agreement with previous reports after an acute transection of the spinal cord the rate of tryptophan hydroxy-l tion was found to be reduced Chlorimipramine reduced the rate of tryptophan hydroxyl tion abov but not below the cute transection. PCPA was by contrast found to equally inhibit the 5-HT synthesi abov and below the transection Pre-treatment with reserpine compl tely antagonized the i hibitory ffect of chlorimipramine on the rate of tryptophan hydroxylation A dose-response tudy revealed th t the magnitude f the reduction i tryptophan hydroxylation induced by the lowest effec-ti dose of chlorimipramine was of the same order as that induced by a sevenfold higher dose and was lso of the same magnitude as the eduction i tryptophan

hydroxylation induced by an acute transection of the spinal ord The concentration of tryptophan in the CNS was largely unaffected by the arious doses of chlor imipramine Taken together these results suggest that the eduction i the synthesis of 5-HT obtained after admi istration of chlorimipramine is mediated mainly i inhibition of the impulse flow in 5-HT neurons

6. A ariety of physi al and psychological forms of stress have previously been reported to increase the synthesis of 5-HT and the concentration of tryptophan i the brain Attempts wer made to clarify whether these biochemical effects of stress are related to the impulse acti ity i 5-HT neurons Male mice were isolated for several weeks i order to induce intensiv fighting when brought together i groups of 20-30 The activity in 5-HT neurons and the rate of tryptophan hydroxylation in the brains of such fighting animal were estimated by determi ing the rate of depl tion of 5-HT after inhibition of tryptophan hydroxylase and the rate of accumulation of 5-HTP after DC inhibition respectively The concentration of tryptophan and the accumulation of 5-HTP i brai wer higher in the fighting animal than i control animals which remained isolated The rate of depletion of 5-HT after inhibition of synthesis was however almost identical i fighting and isolated animals In conclusion the increased synthesis of 5-HT occurring during fighting is probably secondary to the increase in concentration of tryptophan i brain and occurs independently of the impulse acti ity i 5-HT neurons

Andén N E H Corrodi K Fuxe and T Hökfelt Evidence for central 5-hydroxytryptamine receptor stimul tion by lysergic acid diethylamide Br J Pharmac 1968 34 1 7

Andén N E H Corrodi K Fuxe and U Ungerstedt Importance of nervous impulse flow for the neuroleptic induced increase i amine turnover i entral dopamine neurons European J Pharmacol 1971 15 193–199

Andén N E A Dahlström K Fux K Larsson L. Olson and U Ungerstedt Ascending monoami neurons to the telencephalon and diencephalon Acta physiol scand 1966 67 313–326

Andén N E. J Engel and A Rubenson Central decarboxyl tion and uptak of L DOPA Naunyn-Schmiedeberg Arch Pharmacol 1972. 273 11 26

Andén N E K Fuxe and T Hökf lt The importance of th nervous impulse flow for the depl tion of the monoamines from ce tral neurons by some drugs J Pharm Pharmacol 1966 18 630–632.

Andén N E and T Magnusson An improved method for the fluorimetric determination of 5-hydroxytryptamine i tissues Acta physiol scand 1967 69 87 94

Ashcroft G W D E leston and T B B. Crawford 5-Hydroxyindol m tabolism i rat brai A tudy of i termedi te metabolism using the technique of tryptophan loading I J Neurochem 1965 12. 483–492.

Atack C V The determination of dopamine by a modification of th dihydroxyindol fluorimetri assay Br J Pharm. 1973 48 699–714

Atack C and M. Lindqvist Conjoi t nativ and orthophthaldialdehyde-condensate assays for the fluorim tri determin tion of 5-hyd oxyindoles i brain. Naunyn-Schmiedeberg s Arch Pharmacol 1973. 279 267 284

Atack C V and T Magnusson Indi idual lution of noradrenaline (together with adrenali e) dopamine 5-hydroxytryptamin and hi tamine from singl trong cation exchange column by means of mineral acid-organi sol ent mixtures J Pharm Pharmac 1970 22. 625-627

Bartholini G J Constanti idis R. Tissot and A. Pl tscher Formation of monoamines from arious amino acids i the brai after inhibition of extracerebral decarboxylase. Biochem Pharmacol 1971 20 1243-1247

Bartholini G and A Pl tsche Effect of various decarboxylase inhibitors on the central metabolism of dihydroxyphenylalanine J Pharm Pharmac 1969 21 323-324

Becking G C and W J Johnson The inhibition of tryptophan pyrrolase by allopurinol an inhibitor of xanthine oxidase Canad J Biochem 1967 45, 1667 1672

Bédard P A. Carlsson and M. Lindqvist Eff ct of transverse cerebral hemi section on 5-hydroxytryptamine metabolism in the rat brai Naunyn-Schmiedeberg s Arch Pharmacol 1972, 272, 1-15

Bertler Å Effect of reserpine on the storage of catecholamines in brain and other tissues Acta physiol scand 1961 51 75-83

Bertler Å Occurrence and localization of catecholamines i the human brai Acta physiol scand 1961 51 97 107

Bertler Å, A. Carlsson and E Rosengren A method for the fluorimetri determination of adrenaline and noradrenaline i tissues. Acta physiol scand 1958, 44 273-292,

Bertler Å, B Falck C H Owman and E. Rosengren Th localization of monoaminergic blood-brain barrier mechanisms Pharmacol Rev 1966, 18, 369-385,

Bertler Å, and E. Rosengren Occurrence and distribution of catecholami es in the brain Acta physiol scand 1959 47 350-361

Blasberg R. and A Lajtha Substrate specificity of teady-state amino acid transport in mouse brain slices. Arch Biochem Biophys 1965, 112, 361-377

Blaschko H Catecholamine biosynthesis, Br med Bull 1973, 29 105-109

Blaschko H. and W G Levine Metabolism of indolealkylamines In: Handbook of Experimental Pharmacology Eds, O Eichler and A Farah Springer Verlag Berli Heidelberg 1966 19 212 244

Bliss E L. Effects of behavioural manipul tions upon brain serotoni and dopamine In: Serotoni and behavior Eds, J Barchas and E Usdi Academic Press New York 1973 315-324

Bliss E L. J Ailion and J Zwanziger Metabolism of norepinephrine serotonin and dopamine in the rat br i with tress, J Pharmacol exp, Ther 1968 164 122-134

Bogdanski D F H Weissbach and S Udenfiend The distribution of serotoni 5-hydroxytryptophan decarboxylase and monoamine oxidase I brain J Neurochem 1957 1 272-277

Brodie B B. M.S Comer E. Costa and A Dlabac The rol of brain serotonin in the mechanism of the central action of eserpine J Pharm exp Ther 1966 152 340-349

Brodie B B A Pletscher and P A. Shore Evidence that serotonin has a role in brai function Science 1955 122. 968.

Brodie B.B. and W D Reid Serotoni I brains functional considerations Adv Pharmacol 1968 6 B. 97 113.

Brodi B B and P A Shore A concept for the ol of serotonin and norepi nephrine as chemical mediators I the brain Ann N Y Acad Sci 1957 66 631 642.

Brodi H K H R Sack and L Siever Clinical tudies of L 5-hydroxytryptophan i depression In: Ser tonin and behavior Eds J Barchas and E Usdi Academi Press New York. 1973 549-559

Brody Jr J F Behavioral ffects of serotoni depl tion and of p-chlorophenyl alanine(a serotoni depl tor) I rats Psychopharmacologia (Berl) 1970 17 14-33.

Brown B B CNS drug ar tions and I teraction I mice Ar h I t Pharmacodyn 1960 128 391 414

Bruinvels J Inhibition of the biosynthesi of 5-hydroxytryptamine i rat brai by imipramine Europ J Pharmacol 1972 20 231 237

Butcher L L and A.P Di trich Effects on shock-eli ited aggression i mice of preferenti lly protecting brai monoamines against the depleting action of eserpine Naunyn-Schmiedeberg Arch Pharmacol 1973 277 61 70

Butcher L L D L. Rhodes and A Yuwiler Behavioral and biochemical effects of preferentially protecting monoamines I the brai against the action of eserpine European J Pharmacol 1972. 18 204-212.

Carlsson A The occurrence di tribution and physiological role of catecholamines i the nervous system In Symposium on catecholamines Ed O Krayer The Williams and Wilkins Company Baltimore 1959 490-493.

Carlsson A. Drugs which block the storage of 5-hydroxytryptamine and elated amines. In: Handbook of Experimental Pharmacology Eds O Eichler and A Farah. Springer Verlag Heidelberg. 1965 19 529-592

Carlsson A. Basic action of psychoactive drugs Int J Neurol 1967 6 27 45.

Carlsson A. Effect of drugs on amine uptake mechanisms i the brai In: Bayer-Symposium II Springer-Verlag Heidelberg 1970 223-233.

Carlsson A. P Bédard J N Davis W Kehr M. Lindqvist and T Magnusson Physiological control of 5-HT synthesi and turnover i the brai In: Pharmacology and the Future of Man Vol 4: Brain Nerves and Synapses Eds. F E. Bloom and G H Acheson S Karger Basel 1973a. 286-298

Carlsson A. P Bédard M. Lindqvist and T Magnusson The i fluenc of nerve impulse flow on the synthesi and metabolism of 5-hydroxytryptamin i the central nervous system Biochem Soc Symp. 1972a 36 17 32

Carlsson A. H Corrodi K Fuxe and T Hökfelt Effect of antidepressant drugs on the depletion of intraneuronal brai 5-hydroxytryptamine stores caused by 4-methyl-α-ethyl metatyramine European J Pharmacol 1969a 5 357 366

Carlsson A H Corrodi K Fuxe and T Hökfelt Effects of some antidepressant drugs on the depletion of catecholamine stores caused by 4 α-dimethyl-metatyramine European J Pharmacol 1969b 5. 367 373.

Carlsson A. H. Corrodi and B Waldeck Substituierte Dopacetamide als Hemmer der Catechol-O-methyl transferase und der enzymatischen Hydroxylierung aromatischer Aminosäuren In der Catecholamin-Metabolismus eingreifende Substanzen 2. Hel chim Acta 1963 46. 2270-2285

Carlsson A. A. Dahlström K Fuxe and M. Lindqvi t Histochemical and biochemical detection of monoamine elease from brai eurons Lif Sci 1965. 4 809-816.

Carlsson A J N Davi W Kehr M. Lindqvi t and C V Atack Simultaneous measurement of tyrosine and tryptophan hydroxylase activities in brai i iv using an inhibitor of the aromati amino acid decarboxylase Naunyn-Schmiedeberg s Arch Pharmacol 1972b. 275. 153-168.

Carlsson A. B Falck and N Å. Hillarp Cellular localization of brai monoamines. Acta physiol scand 1962 56 Suppl 196 1-28

Bogdanski D F H W Isbach and S Udenfriend The distribution of serotoni 5-hydroxytryptophan decarboxylase and monoamine oxidase in brai J Neurochem 1957 1 272-277

Brodi B B. M.S. Comer E. Costa and A Dlaboc The rol of brain serotonin I the mechanism of the central action of eserpine J Pharm exp Ther 1966 152. 340-349

Brodi B B A Pl tscher and P A. Shore Evidence that serotonin has a rol in brai function Science 1955 122. 968.

Brodi B B and W D Reid Serotonin i brain: functional considerations Adv Pharmacol 1968 6 B. 97 113

Brodi B B and P A Shore A concept for the rol of serotonin and norepinephrine as chemical mediators in the brain Ann N Y Acad Sci 1957 66 631 642.

Brodi H K H R. Sack and L. Siever Cli ical tudies of L 5-hydroxytryptophan in depression In: Serotonin and behavior Eds. J Barchas and E Usdi Academic Press New York. 1973 549-559

Brody Jr J F Behavioral ff ts of serotoni depl tion and of p-chlorophenyl lanine(a serotoni depl tor) i rats Psychopharmacologi (Berl) 1970 17 14-33

Brown B.B CNS drug actions and i teraction i ed Arch. Int Pharmacodyn 1960 128 391-414

Bruinvel J Inhibition of the biosynthesi of 5-hydroxytryptamine i rat brain by imipramine Europ. J Pharmacol 1972. 20 231-237

Butcher L L. and A P Di trich Effects on shock eli ited aggression in mice of preferentially protecting brai monoamines against the depleting action of reserpine Naunyn-Schmiedeberg Arch Pharmacol 1973. 277 61 70

Butcher L L. D L Rhodes and A. Yuwiler Behavioral and biochemical ffects of preferenti lly protecting monoamines i the brai against the action of reserpine European J Pharmacol 1972. 18. 204-212.

Carlsson A. Th occurrence distribution and physiological rol of catecholamines i the nervous system In Symposium on atecholamines Ed O Krayer The Williams and Wilkins Company Baltimor 1959 490-493.

Corrodi H K Fuxe and T Hökfelt A possible role played by central monoamine neurons i thermo- egulation Acta physiol scand 1967 71 224-232

Corrodi H. K Fuxe and T Hökfelt Th effect of immobilization stress on the activity of central monoamine neurons Life Sci 1968 7 107 112.

Curzon G M.H. Joseph and P J Knott Effect of immobiliz tion and food deprivation on r t brai tryptophan metabolism J Neurochem 1972 19 1967 1974

Dahlström A. and K Fuxe Evidence for the existence of monoamine neurons i the cell bodies of brai stem neurons Acta physiol scand 1965 62. Suppl 232. 1 55.

Dahlström A. J. Häggendal and C Atack Localization and transport of serotonin. In: Serotonin and behavior Eds. J Barchas and E Usdin Academi Press New York 1973. 87 96

Eccleston D G W Ashcroft and T B B Crawford 5-Hydroxyindole metabolism i rat brai A study of i termediate metabolism using the technique of tryptophan loading. II J Neurochem 1965. 12 493-503.

El H. and R. Ti i Effect of forced motility on noradrenaline and 5-hydroxytryptamine metabolism i different parts of the brai Psychopharmacologi (Berl) 1972 26. 195-200.

Engel J and K Modigh Tryptophan-induced suppression of conditioned avoidanc behavior i rats. In: Advances i biochemical psychopharmacology Vol 10 and 11 Serotoni new istas Eds E Costa and M. Sandler Raven Press New York. 1974 (In press)

Erspamer V Pharmacology of indolealkylamines Pharmacol Rev 1954 6 425-487

Falck B N Å Hillarp G Thiem and A. Torp Fluorescence of catecholamines and related compounds condensed with formaldehyde J Histochem Cytochem 1962. 10 348-354

Fernstrom J D and R.J Wurtman Brai serotoni content: Physiologi al dependence on plasma tryptophan lev ls. Science 1971 173. 149 151

Fernstrom J D and R.J Wurtman Brai serotoni content: Physiological regulation by plasma neutral amino acids. Science 1972. 178 414-416

Fog R. Rage reactions produced in rats by combination of thymoleptics and monoamine oxidase i hibitors Pharmacol Res Comm 1969 1 79-82

Fuxe K L.L Butcher and J Engel DL 5-hydroxytryptophan-induced changes I central monoaml neurons after peripheral decarboxylase inhibition J Pharm Pharmac 1971 23, 420-424

Fuxe K T Hökfelt and U Ungerstedt Morphological and functional aspects of central monoamine neurons I t Rev Neurobiol 1970 13, 93-126

Gaddum J H Drugs antagonistic to 5-hydroxytryptamine In CIBA Foundation Symposium on Hypertension Humoral and Neurogenic Factors Littl Brown & Company 1954 75-77

Gál E M. and S.A Millard The mechanism of inhibition of hydroxylases in ivo by p-chlorophenyl lanine: the effect of cycloheximide Biochim Biophys. Acta. 1971 227 32-41

Gál E M. A. Roggeveen and S A Millard DL [2-^{14}C]p-Chlorophenyl lanine as an inhibitor of tryptophan 5-hydroxylase J Neurochem 1970 17 1221 1235

Giacalone E. M. Tansell L V l elli and S Garatti I Brain serotonin metabolism I isol ted aggressive mi Biochem Pharmacol 1968 17 1315-1327

Grahame-Smith D G Tryptophan hydroxylation I brai Biochem Biophys. Res. Commun 1964 16, 586-592.

hame-Smith D G The biosynthesis of 5-hydroxytryptamine I brai Biochem J 1967 105, 351

Grahame-Smith D G Studies i ivo on the relationship between brain tryptophan br i 5-HT synthesi and hypera ti ity i rats treated with a monoami e oxidase i hibitor and L tryptophan J Neurochem 1971 18, 1053-1066

Grahame-Smith D G Does the total turnover of brai 5-HT eflect the functional activity f 5-HT i brai ? In: Serotoni and behavior Eds J Barchas and E. Usdi Academi Press New York 1973 5-7

Grahame-Smith D G and A. G Parfitt Tryptophan transport across the synaptosomal membrane J Neurochem 1970 17 1339-1353

Greengard O and P Feigelson The purification and properties of liver tryptophan pyrrolase J biol Chem 1962 237 1903-1907

Guroff G and W Lovenberg Metabolism of aromatic amino acids. In: Handbook of Neurochemistry Vol III Ed A Lajtha Plenum Press New York London 1970 209 223.

Guroff G and S Udenfriend Studies on aromatic amino acid uptak by rat brain in ivo J biol Chem 1962. 237 803-806

Hagen B P and L H. Cohen Biosynthesi of indolealkylamines. Physiological release and transport of 5-hydroxytryptamine In Handbook of Experimental Pharmacology Eds O Eichler and A Farah Springer Verlag Heidelberg. 1966. 19 182-211

Hamon M. S Bourgoin and J Glowinski Feedback regulation of 5-HT synthesis in r t stri tal slices. J Neurochem 1973 20 1727 1745.

Hartman E. R. Chung and C p Chien L Tryptophan and sleep P ychopharmacologia (Berl) 1971 19 114-127

Heller A. and R Y Moore Effect of central nervous system lesions on brain monoamines in the rat J Pharm exp Ther 1965. 150 1-9

Hess S M. B.G Redfield and S Udenfriend The effect of monoamine oxidase inhibitors and tryptophan on the tryptamine content of animal tissue and urine J Pharmacol exp Ther 1959 127 178-181

Horita A. and A.E Hamilton P tentiation of the central actions of 5-hydroxytryptophan in rabbits by DL-α-hydrazino-α-methyldopa J Pharm Pharmac 1970 22. 389-391

Häggendal J and T Malmfors The effect of nerve stimul tion on catecholamines taken up i adrenergi nerves after reserpine pretreatment Acta physiol scand 1969 75 33-38

Hökfelt T K Fuxe and M. Goldstei Immunohistochemical localization of aromatic L-amino acid decarboxylase (DOPA decarboxylase) i entral dopamine and 5-hydroxytryptamine nerv cell bodies of the rat Brai Research 1973. 53 175-180

Iversen L L Neuronal uptak processes for amines and amino acids. In Advances i Biochemical Psychopharmacology Vol 2 Eds E. Costa and E. Giacobini Raven Press New York. 1970 109-132.

Jéquier E W Lovenberg and A. Sjoerdsma Tryptophan hydroxylase inhibition the mechanism by which p-chlorophenylalanine depl tes rat brai serotoni Mol Pharmacol 1967 3 274-278.

Jéquier E. D S Robinson W Lovenberg and A. Sjoerdsma Further tudies on tryptophan hydroxylase i rat br i stem and beef pineal Biochem Pharmacol 1969 18. 1071-1081

Fuxe K. L L. Butcher and J Engel DL 5-hydroxytryptophan-induced changes I central monoamine neurons after peripheral decarboxylase inhibition J Pharm Pharmac 1971 23. 420-424

Fuxe K T Hökfelt and U Ungerstedt Morphologi al and functional aspects of central monoamin neurons Int Rev Neurobiol 1970 13 93-126.

Gaddum J H Drugs antagoni tic to 5-hydroxytryptamine In: CIBA Foundation Symposium on Hypertension Humoral and Neurogenic Factors Littl Brown & Company 1954 75-77

Gál E M. and S A Millard The mechanism of inhibition of hydroxylases I ivo by p-chlorophenyl lanine: the effect of cycloheximide Biochim Biophys. Acta 1971 227 32 41

Gál E M. A. Roggeveen and S A Millard DL [2-^{14}C]p-Chlorophenyl lanine as an inhibitor of tryptophan 5-hydroxylase J Neurochem 1970 17 1221 1235.

Giacalone E M. Tansell L. V Izelli and S Garatti I Brain ser toni metabolism I isolated aggreal ml Biochem Pharmacol 1968 17 1315-1327

Grahame-Smith D G Tryptophan hydroxylation I brai Biochem Biophys Res Commun 1964 16 586-592.

Smith D G The biosynthesis of 5-hydroxytryptamine I brai Biochem J 1967 105 351

Grahame-Smith D G Studies I ivo on the rel tionship between brai tryptophan br I 5-HT synthesi and hyperactI ity I rats treated with a monoamine oxidase I hibitor and L tryptophan J Neurochem 1971 18. 1053-1066

Grahame-Smith D G Does the total turnover of brai 5-HT reflect the functional activity of 5-HT I brai ? In: Serotonin and behavior Eds. J Barchas and E. Usdi Academi Press New York 1973 5-7

Grahame-Smith D G and A. G Parfitt Tryptophan transport across the synaptosomal membrane J Neurochem 1970 17 1339-1353.

Greengard O and P Feigelson The purification and properties of liver tryptophan pyrrolase J biol Chem 1962 237 1903-1907

Guroff G and W Lovenberg Metabolism of aromatic amino acids. In: Handbook of Neurochemi try Vol III Ed A. Lajtha Plenum Press New York London 1970 209 223.

Lloyd K G and O Hornykiewicz Occurrence and distribution of aromatic L amino acid (L-DOPA) decarboxylase I the human brain J Neurochem 1972, 19 1549-1559

Lovenberg W Tryptophan hydroxylase and serotonin synthesis in the brai In: Pharmacology and the Future of Man Vol 4: Brain Nerves and Synapses Eds. F E Bloom and G.H Acheson S Karger Basel 1973, 222 244

Lovenberg W H Weissbach and S Udenfriend Aromatic L amino acid decarboxylase J biol Chem 1962 237 89-93

Lycke E. K Modigh and B E Roos The monoamine metabolism in iral encephalitides of the mouse I Virological and biochemical results Brain Research, 1970 23, 235-246.

Macon J B L Sokoloff and J Glowinski Feedback control of rat brai 5-hydroxytryptamine synthesi J Neurochem 1971 18 323-331

Malmfors T Studies on adrenergic nerves, Act physiol scand 1965, 64 Suppl 248 58-61

Mantegazzoni P Pharmacological actions of indolealkylamines and precursor aminoacids on the central nervous system In: Handbook of Experimental Pharmacology Eds. O. Eichler and A. Farah Springer Verlag Heidelberg 1966.XIX. 424-470

Matthew H.J S The management of self poisoning due to clomipramine (Anafranil) J Int Med Res 1973 1 485-488.

McCaman R.E M.W McCaman J M. Hunt and M.S Smith Microdetermination of monoamine oxidase and 5-hydroxytryptophan decarboxylase acti ities i nervous tissues J Neurochem 1965 12, 15-23

McGeer E.G. and D A V Peters In itro screen of inhibitors of rat brain serotonin ynthesi Can J Biochem 1969 47 501 506.

McKenzi G M. Apomorphine-induced aggression in the at Brain Research 1971 34 323-330

Meek J L. and K Fuxe Serotonin accumul tion after monoamine oxidase inhibition Effect of decreased impulse flow and of some antidepressants and hallucinogens Biochem Pharmacol 1971 20 693-706.

Meek J K Fux and N E. Andén Effects of antidepressant drugs of the imipramine type on central 5-hydroxytryptamine neurotransmission. European J Pharmacol 1970 9 325-332

Meek J L. and N H Neff Tryptophan 5-hydroxylase: approximation of half lif and rate of axonal transport J Neurochem 1972. 19 1519-1525

Meek J and B Werdl lus Hydroxytryptamine turnover decreased by the anti depressant drug chlorimipramine J Pharm Pharma 1970 22. 141 143

Moir A T B and D Eccleston The effect of precursor loading in the cerebral metabolism of 5-hydroxyindoles J Neurochem. 1968 15. 1093-1108.

Murphy D L M. Baker J K ti and W E. Bunney Jr Behavioral and metaboli effects of L tryptophan in unipolar depressed patients In: Serotoni and behavior Eds. J Barchas and E Usdin Academi Press New York 1973. 529 537

Neff N H and E. Costa Application of steady-state kinetics to the tudy of catecholamine turnover after monoamine oxidase inhibition or reserpine admini tration J Pharm exp Ther 1968 160 40-47

Neff N H and T N Tozer In lv measurement of brain ser toni turnover Adv Pharmacol 1968 6 A 97 109

Nemeth A. M. The effect of 5-fluorouracil on the developmental and adaptive formation of tryptophan pyrrolase J biol Chem 1962. 237 3703-3706

Pl tscher A. Monoamine oxidase inhibitors. Pharmacol Rev 1966 18 121 129

Porter C C L S Watson D C Titus J A. Totaro and S S Byer Inhibition of DOPA decarboxylase by the hydrazino analog of α-methyldopa Biochem Pharmacol 1962. 11 1067 1077

Randrup A. and L Munkvad Relation of br l catecholamines to aggressiveness and other forms of behavioral ex itation In: Aggressive behavior Eds. S. Garatti l and E. B Sigg Wiley and Excerpta Medica Foundation New York Amsterdam 1969 228-236

Rapert M. M. Serum vasoconstri tor (serotoni) V The presence of creatinine l the complex. A proposed tructure of the vasoconstrictor principle. J biol Chem 1949 180 961-969

Rapert M. M. A A. Green and I H Page Serum vasoconstri tor (serotoni) IV Isol tion and characterization J biol Chem 1948 176. 1243-1251

Roos B. E and R. Sjöström 5-Hydroxyindoleacetic acid (and homovanilli acid) levels i the cerebrospinal fl id after probenecid application in patients with mani -depressiv psychosis. Pharmacol Clin. 1969 1 153-155

van Rossum J B Mode of action of psychomotor stimulant drugs In: Int Rev Neurobiol Eds C C Pf iffer and J R. Smythies. Academi Press New York. 1970. 12. 307 383.

Rubenson A. Further studies on the mechanism of the central hypotensive effect of L-dopa DL-m-tyrosine and L-α-methyldopa J Pharm Pharmac 1971 23. 228-230.

Schildkraut J J S M. Schanberg G R. Breese and I J Kopi Effects of psychoactive drugs on the metabolism of i tracisternally admi istered serotoni i rat brain. Biochem Pharmacol 1969 18. 1971 1978.

Schubert J Effect of spinal transection on the metabolism of 5-hydroxyindoles formed i ivo from ^{3}H-tryptophan in the rat pinal cord Acta physiol scand 1973. 87 557 566.

Schubert J H. Nybäck and G Sedvall Effect of antidepressant drugs on accumulation and disappearance of monoamines formed i ivo from labelled precursors in mouse brai J Pharm Pharmac 1970 22 136-139

Sharman D F The catabolism of catecholamines Br med Bull 1973. 29 110-115.

Sheard M.H. A. Zolovick and G K. Aghajanian Raphe neurons Effect of tricycli antidepressant drugs Brain Research 1972. 43 690-694

Shields P J and D Eccleston Effects of lectrical stimulation of rat midbrai on 5-hydroxytryptamine synthesis as determined by a sensitiv radioisotope method J Neurochem 1972. 19 265-272

Simmons A V D Cox and E J Ross Case of self poisoning with multipl antidepressant drugs Lancet 1970 1 214-216

Sjoerdsma A. T F Smith T D Stevenson and S Udenf iend Metabolism of 5-hydroxytryptamine (serotoni) by monoamine oxidase Proc Soc. Exp. Biol Med 1955 89 36-38

Sjöström R. and B E. Roos 5-Hydroxyindoleaceti acid and homovanillic acid in erebrospinal fluid in mani -depressiv psychosis Europ J clin. Pharmacol 1972. 4 170-176.

Smith A.D Mechanisms involved in the release of noradrenaline from sympathetic nerves Br med Bull 1973 29 123-129

Smith C.B and P B Dews Antagonism of locomotor suppressant effects of reserp i mice Psychopharmacologia. 1962. 3. 55-59

Meek J L and N H Neff Tryptophan 5-hydroxylase: approximation of half-lif and rate of axonal transport J Neurochem 1972. 19 1519-1525

Meek J and B Werdinius Hydroxytryptamine turnover decreased by the anti-depressant drug chlorimipramine J Pharm Pharma 1970 22, 141 143.

Moir A.T B. and D Eccleston The effect of precursor loading in the cerebral metabolism of 5-hydroxyindoles J Neurochem 1968 15, 1093-1108.

Murphy D L M. Baker J Kott and W E. Bunney Jr Behavioral and metaboli effects of L tryptophan I unipolar depressed patients In: Serotoni and behavior Eds J Barchas and E Usdi Academic Press New York 1973 529-537

Neff N H and E Costa Application of steady-state kinetics to the study of catecholamine turnover after monoamino oxidase inhibition or reserpine administration J Pharm exp Ther 1968 160 40-47

Neff N H and T N Tozer I vo measurement of brai serotoni turnover Adv Pharmacol 1968 6 A 97 109

Nemeth A.M. The effect of 5-fluorouracil on the developmental and adaptive formation of tryptophan pyrrolase J biol Chem 1962. 237 3703-3706

Pl tscher A. Monoamine oxidase inhibitors. Pharmacol Rev 1966. 18 121 129

Porter C C L.S Watson D C. Titus J A. Totaro and S S Byer Inhibition of DOPA decarboxylase by the hydrazino analog of α-methyldopa Biochem Pharmacol 1962. 11 1067 1077

- kandrup A and L. Munkvad Rel tion of brai catecholamines to aggressiveness *and ther forms of behavioral excitation In: Aggressiv behavior Eds.* S Garattini and E.B Sigg Wiley and Excerpta Medica Foundation New York Amsterdam 1969 228-236

Raport M.M. Serum vasoconstri tor (serotoni) V The presence of creatinine I the complex. A proposed tructure of the asoconstri tor principle J biol Chem 1949 180 961 969

Raport M.M. A.A Green and I H. Page Serum vasoconstri tor (serotonin) IV Isolation and characterization. J biol Chem 1948. 176. 1243-1251

Roos B E. and R. Sjostrom 5-Hydroxyindoleacetl acid (and homovanilli acid) level I the cerebrospinal fluid after probenecid application I patients with manic-depressi psychosis. Pharmacol Cli 1969 1 153-155

Udenfriend S J G Chri tenson and W D irman Decarboxylation of 5-hydroxy-tryptophan In Pharmacology and the F ture of Man Vol 4 Brain Nerves and Synapses Eds F E Bloom and G H Acheson S Karger Basel 1973. 245-256.

Udenfriend S E. Titus H Weissbach and R.E Peterson Biogenesis and metabolism of 5-hydroxyindole compounds J biol Chem 1956. 219 335-344

Udenf iend S and H W issbach Turnover f 5-hydroxytryptamine (seroton) i tissues. Proc. Soc. exp Biol (N Y) 1958 97 748-751

Udenfriend S H. W issbach and D F Bogdanski Increase i tissue serotoni following admi istration of its precursor 5-hydroxytryptophan J biol Chem 1957 224 803-810

Ungerstedt U Stereotaxi mapping of the monoamine pathways in the rat brain. Acta physiol scand 1971 Suppl 367 1 48

Valzelli L Aggressive behavior induced by isol tion In: Proc Int Symp on the Biology of Aggressi Behavior Eds S Garatti i and E B Sigg. Excerpta Medical Foundation Amsterdam 1969 70-76.

V lzelli L. The isol tion syndrome in mice Psychopharmacologi (Berl) 1973 31 305-320

Vogt M. The concentration of sympathin i different parts of the central nervous system under normal conditions and after the admi i tration of drugs J Physiol 1954 123. 451-481

Woolkes T P and S Udenfriend A fluorimetric method for the estimation of tyrosine i plasma and tissues J Lab cli Med. 1957 50 733-736.

Weiner N Regulation of norepinephrine biosynthesis. Ann Rev Pharmacol 1970 10 273-290

Weissbach H. W King A Sjoerdsma and S Udenfriend Formation of indole-3-aceti acid and tryptamine i animals. J biol Chem 1959 234 81-86.

Welch A.S and B L. Welch Effect of tress and para-chlorophenylalanine upon brai serotoni 5-hydroxyindoleaceti acid and catecholamines in grouped and isolated mice Biochem Pharmacol 1968a. 17 699-708

Welch B L and A.S Welch Greater lowering of brai and adrenal catechol amines i group-housed than i individually-housed mice admi istered DL α-methyltyrosine J Pharm Pharmacol 1968b 20 244-246

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 404

A SPECTROPHOTOMETRIC METHOD OR ANALYSIS OF OXYGEN CONSUMPTION IN VITRO ON THE MICROSCALE

BY

RAGNAR HULTBORN

Kjell Modigh Functional aspects of 5-Hydroxytryptamine turnover in the Central Nervous System

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 404

From Institute of Neurobiology and Department of Histology
University of Göteborg, Sweden

A SPECTROPHOTOMETRIC METHOD FOR ANALYSIS OF OXYGEN CONSUMPTION IN VITRO ON THE MICROSCALE

BY

RAGNAR HULTBORN

This thesis is partly based on the following publications

1 Ragnar Hultborn A sensitive method for measuring oxygen consumption *Anal. Biochem.* 47 442–450 1972

2 Hans Herlitz and Ragnar Hultborn A microspectrophotometric technique for determination of respiration in comparison to the Cartesian diver method. – Respiratory activity of rat corpus luteum cells with reference to substrate *Acta physiol. scand.* In press.

3 Ragnar Hultborn and Holger Hydén Microspectrophotometric determination of nerve cell respiration at high potassium concentration. *Exp Cell Res.* submitted for publication

4 Ragnar Hultborn, Sune Larsson and Lennart Pilhage An automatic cuvet changer for spectrophotometric oxygen consumption measurements and for conventional spectrophotometry *Zeiss Informationen* accepted for publication

5 Ragnar Hultborn and Sante Olling Studies on leucocyte function by measuring respiration and nitroblue tetrazolium reduction by simplified methods *Scand J Clin. Lab Invest* 32, 297–304 1973

Printed in Sweden by Gotab Kungälv 1974

Contents

Introduction 5

Theory of the *in vitro* spectrophoto-respirometric system 7
- Absorbance characteristics of hemoglobin solutions 7
- Oxygen-hemoglobin dissociation kinetics 8
- The O_2-content in a closed system as related to oxygen tension time and hemoglobin concentration 9
- Calculation of O_2-consumption from an absorbance shift curve (OD-curve) 11

Hemoglobin solutions 14
- Preparation and storage 14
- Characterization 14

Determination of O_2-consumption in the range of 1 μl O_2/h 16
- Incubation chambers (cuvets) 16
- Analysing equipment 17
  - Basic unit 17
  - Modifications 17
  - Working characteristics 19
- Procedure 20
- Differential-spectrophoto-respirometry 21
- Accuracy 23
- Final remarks 23

Determination of O_2-consumption between 10^{-2} and 10^{-4} μl O_2/h 24
- Incubation chambers (cuvets) 24
  - Chambers used for O_2-uptake in the range of 10^{-2} μl/h 24
  - Chambers used for O_2-uptake in the range 10^{-4} – 10 μl/h 25
- General detecting equipment 27
  - Light source 27
  - Monochromator 28
  - Microscope and optics 29
  - Photometer head and detector with amplifier 29
  - Recorder 29
- Special equipment 29
  - Temperature regulation of the object 29
  - Automatic cuvet changing equipment 30
- Procedure 31
  - Loading of chambers drilled in object slides 31
  - Loading of sapphire chambers 32
- Addition of reactants in the course of an experiment 34

General discussion 36
Similarities and dissimilarities to other microrespirometric methods with reference to O_2 tension 36
Sensitivity resolution and accuracy 38
Other considerations specific for the spectrophoto-respirometric system 39
Facility and versatility 40
Applications 41

Summary 42

Acknowledgements 43

References 44

Introduction

Metabolism is fundamental for cell life and is dependent on a chain of enzymic processes, the energy required mainly being supplied by mitochondrial oxidative activity Hence analysis of oxygen consumption constitutes an important approach to studies of how enzymic reactions are affected in normal or abnormal cells by changes in their functional activity as well as in their chemical or physical environment. It must be recognized that results from investigations on gas exchanges represent a cellularly integrated allover measure of oxidative activity though intermediate reactions may be studied using specific substrates or metabolic inhibitors. This integrated functional activity often considerably differs from the potential activity of a cell as indicated by its enzyme and metabolite content.

Due to cellular heterogeneity of almost any tissue metabolic studies must often be performed on single cells or on a tiny tissue sample In such cases it is a prerequisite to have access to sufficiently sensitive methods. The present work was designed to evolve a sensitive convenient method capable of measuring oxygen consumptions ranging from 1 μl/h to 10^{-6} μl/h The principle of the technique is to incubate cells or tissue samples in oxyhemoglobin solutions in minute cuvets. The absorbance shift of the incubate during hemoglobin reduction is then recorded spectrophotometrically

Manometric methods for estimating exchange of gases on the macroscale have been used for a long time a wide variety of techniques being employed. One type of respirometer which has met with wide use is commonly called the "Warburg" instrument, although as pointed out by Warburg (1926) it was used before his adoption of it. The respirometer is based on the principle that at constant temperature and gas volume any change in amount of gas can be measured by changes in its pressure A detailed review of manometric procedures for macroscale investigations is given in Umbreit *et al.* (1972) Several micro-adaptations of manometric procedures have been presented, though the Cartesian diver principle adopted by Linderstrom-Lang (1937) proved to be the one most commonly used Adaptations of this procedure have led to the possibility of analyses of gas exchange in the range of 10^{-4} to 10^{-5} μl/h (*c f* Holter and Zeuthen 1966) Recently Oman and Brzin (1972) published a modification of the Cartesian diver principle in essence not manometric where the authors claim to have a sensitivity of 10^{-6} μl/h

Though the theory of polarographic analysis of oxygen tension was published some 50 years ago (Heyrovsky 1923) it remained essentially unused until the past decade (*c f* Lessler and Brierly 1969). Polarographic instruments have now been modified for microscale work and are widely employed for determinations of oxygen tension *in vivo* (Payne and Hill 1966 Lübbers 1969) Oxygen electrodes are however not suited for O_2-uptake studies on a single cell level since technical difficulties would arise due to the inevitably minute incubation chambers. Furthermore the electrodes have an intrinsic consumption of oxygen which might be of significance on this scale of work.

The advent of spectrophotometry in the early nineteenth century soon led to the application of this technique in the field of biological sciences. Simultaneously with the increasing knowledge of the spectral characteristics of hemoglobin and studies on tissue

metabolism, Vierordt as early as 1875 made the observation that the spectrum of reflected light from the skin of the hand changed from having two absorbing bands in the visible light to only one broader band upon occlusion of the blood supply to the hand. Vierordt explained this phenomenon correctly as being the expression of tissue metabolism, reducing the oxyhemoglobin within the skin Vierordt (1878) and his pupil Dennig (1883) further reported on this reaction but the observations were then forgotten for almost half a century when Kahn (1922) further developed these *in vivo* investigations. Since then a fairly large number of reports on tissue metabolism *in vivo* studied by either reflection or transmission photometry have appeared (*e.g.* Meyer and Reinhold 1926 Koch 1928 Nicolai 1932 Wodick and Lübbers 1973) During the same period an ever increasing number of investigations on spectrophotometric determination of hemoglobin saturation of blood appeared (*c f* Nilsson 1960) procedures which to a large extent replaced the laborious gas extraction techniques (e.g. Van Slyke 1924). Spectrophotometric approaches for metabolic investigations *in vitro* using hemoglobin as indicator of oxygen tension have only recently been reported. Arvanitaki and Chalazonitis (1949) found a hemoprotein situated intracellularly in the giant neurons of *Aplysia* and *Helix*. The degree of oxygen saturation of this hemoglobin compound could be recorded microspectrophotometrically and be related to the O_2 tension of the environment (*c f* Chalazonitis 1968) Bârzu and Borza (1967) Bârzu *et al.* (1968) Bârzu and Satre (1970) and Bârzu *et al.* (1972) described in a series of excellent investigations quantitative measurements of oxidative activity of mitochondrial suspensions in "standard" cuvets containing diluted oxyhemoglobin solutions The use of cytochromes in oxidation reduction experiments especially fast reactions have been [illegible]lished by e.g. Chance (*c f* 1965) though these investigations did not aim at [illegible]titative O_2 uptake determinations.

Theory of the in vitro spectrophoto-respirometric system

The microrespirometric procedure described in this work is based on spectrophotometric recording of the absorbance change of an oxyhemoglobin solution when reduced due to the presence of respiring material within a sealed cuvet. The aim of the methodological development was to design a procedure having a large sensitivity range especially extending so far in the microscale that the respiratory activity of single small cells could be measured. This demanded use of extremely small cuvets, also referred to as incubation chambers, to decrease the amount of available oxygen, in physical solution as well as bound to the hemoglobin. A decrease in amount of O_2 could also be accomplished by use of low pigment (*i.e.* hemoglobin) concentrations.

Several physico-chemical relationships determining the functional properties of such a system must be considered for correct interpretation of the results. Below a discussion of the most important ones is presented.

Absorbance characteristics of hemoglobin solutions.

Not long after the introduction of spectrophotometry the technique was used in the study of hemoglobin. In 1862 Hoppe published his observations of absorption bands from oxyhemoglobin in the green and yellow part of the spectrum. Stokes (1864) recognized spectrophotometrically the reversibility of oxygenated and reduced hemoglobin. In 1873 Vierordt and in 1880 MacMunn published extensive monographs on the use of spectroscopes, the latter dealing primarily with the absorption spectra of hemoglobin and its numerous derivatives. Soret (1878, 1883), Arsonvales (1890) and Gamgee (1897) were the first to report the spectral characteristics in the blue violet and ultra-violet regions. During this century an ever increasing amount of literature on the absorption spectra of hemoglobin and its derivatives has appeared and recently Assendelft (1970) published a detailed monograph from which extinction data used in this work have been obtained (Fig. 1).

In the respirometric system considered here measurements are made on solutions of oxygenated and deoxygenated hemoglobin in various proportions, the solutions generally being rather dilute compared to whole blood (dilution 1/20 to 1/50), and the optical pathlengths being short (1 mm to 0.1 mm). To obtain sufficient absorption it is necessary to select a wavelength where the extinction coefficients are high, *i.e.* it is not advantageous to choose the red part of the spectrum generally used for analyses of O_2 saturation in whole blood (*in vivo* or *in vitro*). Measurements in the blue region however yield sufficiently low transmission for the present purpose. Another requirement is that a region of the spectrum be selected where the extinction coefficient of reduced and oxygenated hemoglobin differ preferably to a large extent. A wavelength region fulfilling the above mentioned demands exists at 435 nm. In this region the slopes of the two extinction curves $(\frac{d\,\varepsilon}{d\,\lambda})_{435}$ are similar (Fig. 1), thus a change of half width of the monochromatic light used (see below) will not drastically influence the amplitude

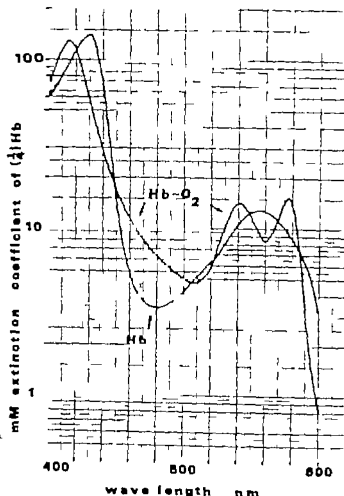


Fig. 1 Extinction-wavelength curves for oxygenated and reduced hemoglobin. Any wavelength region where there is a great difference between extinction coefficients of the two types of hemoglobin can be used for recording of absorbance change during pigment reduction. Millimolar extinction coefficients for one heme and globin moiety (1/4) Hb are given. (Data obtained from Assendelft 1970)

the measured difference in absorption, *i.e.* the change in optical density (OD) between oxygenated and reduced hemoglobin. The relative difference in absorption at 435 nm (2.5 times) is exceeded only in the red part of the spectrum. Extinction coefficients of oxygenated, $\epsilon_{(1/4)HbO_2}$ and reduced hemoglobin $\epsilon_{(1/4)Hb}$ at 435 nm, is 41×10^3 and 115×10^3 respectively (Fig. 1)

Oxygen-hemoglobin dissociation kinetics.

The complex nature of the reversible binding of oxygen to hemoglobin was first reported by Bohr (1904) who noticed a non-linearity in the equilibrium $Hb_4 + 4O_2 \rightleftarrows (HbO_2)_4$ The sigmoid relationships between hemoglobin saturation and oxygen tension was mathematically analysed by Hill (1910) Adair (1925) *and others and today the complex* molecular interactions during oxygen binding are under intense investigation (*c f* Rorth and Astrup 1972, Rorth 1973) Several different approaches to the experimental determination of the oxygen dissociation curve have been devised. Discontinuous recording of this curve was done by tonometry (letting the solution come in equilibrium

with a gas mixture of known composition) followed by gasometric determination of the oxygen content in the solution (e.g. Roughton 1963) Discontinuous tonometry followed by spectrophotometric determination of the hemoglobin saturation was also performed (e.g. Benesch *et al* 1965) During the past two decades continuous recordings of the oxygen dissociation curve have been made by use of spectrophotometry in combination with a gradual change of the gas mixture with which the solution is in equilibrium (e g Sick and Gersonde 1969) Similarly the simultaneous use of oxygen electrodes and spectrophotometry combined with an oxygen consuming and producing system which changes the O_2 tension in the solution has been introduced (Colman and Longmuir 1963 Longmuir and Chow 1970)

It was early observed that the equilibrium between oxygen and hemoglobin was influenced by various physico-chemical factors such as hemoglobin concentration, temperature pH carbon dioxide and several organic compounds During the past decade an immense interest has been focussed on the action of the organic phosphate compounds 2,3-diphosphoglycerate and inositol hexaphosphate (phytic acid) since these physiologically occuring substances (the former in mammals, the latter in birds) drastically decrease the oxygen affinity of hemoglobin (Benesch and Benesch 1967 Chanutin and Curnish 1967 Benesch *et al.* 1968 Gibson and Gray 1970 *c f* Kilmartin and Rossi-Bernardi 1973)

The most important characteristics of the dissociation kinetics of a hemoglobin solution is the half-saturation value (P_{50}) *i.e* the oxygen tension at which 50 % of the hemoglobin is oxygenated and the slope of the dissociation curve at its linear part. This slope can be expressed in various ways and in this work it is denoted by the change in hemoglobin saturation (% HbO_2) per unit change in oxygen tension $\frac{\Delta \% HbO_2}{\Delta P_{O_2}}$ *In vitro* P_{50} is generally between 20 and 30 mm Hg and the slope is ~ 0 02 mm Hg^{-1} In the *in vitro* system presented in this work it will be noticed that the P_{50} value is decreased and the slope is increased due to hemoglobin dilution and (partial) stripping" of hemoglobin from its phosphate compounds (Berman *et al* 1971) The half saturation value is of importance when considering the environment in which cellular respiration is measured in the procedure described in this work (see General Discussion) Further it is necessary to know the slope of the dissociation curve for the calculation of oxygen uptake in the system (see below)

The O_2-content in a closed system as related to oxygen tension time and hemoglobin concentration.

The oxygen present in a hemoglobin solution is divided into two pools, one physically dissolved and the other bound to the hemoglobin The relative amounts of these pools are dependent on the pigment concentration. When this is low the former pool will dominate when high the latter

The amount of oxygen in physical solution is directly proportional to the oxygen tension, but the pool combined with the hemoglobin is not, as discussed above The total amount of oxygen in the chamber is thus nonlinear with regard to O_2 tension since it is the sum of one linear and one nonlinear function (Fig. 2)

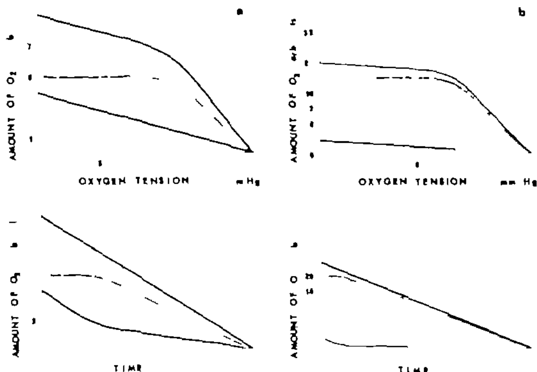


Fig. 2. In this figure the graphs are schematically drawn fo an arbitrarily chosen hemoglobin concentration as well as for a certain physicochemical environment. The amou ts of physically dissolved oxygen, of hemoglobin-bound oxygen and the sum f these two fractions in a closed system related to oxygen tension (uppe diagram in (a) and (b)) or time (lower diagram in (a) and (b)). In) the hemoglobin concentration is four times of that in (a) The upper solid curves in all diagrams represent the total amount of oxygen, the lower solid ones represent the physically dissolved pool and the d tted curves depict the hemoglobin-bound fraction. Note the nonlinear transformation of the graphs on substituting the independent variable time for tension and the increased proportion of the hemoglobin-bound oxygen at increased hemoglobi concentration. It can be seen that the change in shape of the dotted curves between upper and lower diagrams is less pronounced in (a), *i.e.* at low hemoglobin concentration. At very low hemoglobin concentration there will be virtually no change in shape of the curve

Assuming constantly respiring material within the closed chamber the total amount of oxygen will decrease linearly when plotted against time The function of the total O_2-content has thus been nonlinearly transformed when substituting the independent variable time for O_2 tension. Since this transformation does not affect the proportions of the subfunctions (physically dissolved and hemoglobin bound O_2) at any arbitrary tension or time these functions can easily be reconstructed with time as the independent variable (Fig. 2). When the hemoglobin concentration is high the function for the relationship between time and the hemoglobin bound oxygen called f(t) will be linear (Fig. 3) *i.e.*

$$\lim_{[Hb] \to \infty} f(t) = k \cdot t$$

On the other hand when the concentration approaches zero the function f (t) will be

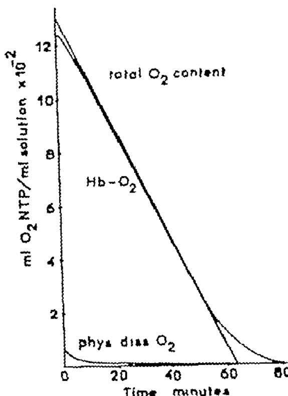


Fig. 3 Data from an actual experiment on the respiration of yeast cell in a microincubation chamber the hemoglobin concentration being 1.36×10^{-3}M It is seen that the absorbance shift curve is almost linear with time and that the physically dissolved fraction can be disregarded The asymptotic approach to the abscissa is due to final cellular hypoxia. NTP normal temperature pressure (From Hultborn 1972)

identical to the function called f (p) describing the relationship between the O_2 tension and the hemoglobin bound oxygen

$$\lim_{[Hb] \to 0} f(t) = f(p)$$

The graph describing the relationship between hemoglobin bound O_2 (i.e. O_2-saturation) and time is the one recorded spectrophotometrically during the experiment

Calculation of O_2-consumption from an absorbance shift curve (OD-curve).

A detailed description of the treatment of the spectrophotometrically obtained OD-curve based on the considerations above will be given in this section In Fig. 4 the same graph as in Fig. 2 is presented the dotted curve corresponding to an experimentally achieved curve and in Fig. 5 an actual recording is illustrated for comparison The initial part is nonlinear due to the sigmoid shape of the dissociation curve and the final part has an asymptotic approach to the "reduced level" partly due to cellular hypoxia. For calculations the linear steepest, part of the OD-curve corresponding to the region of half saturation is used. In this region the slope of the curve $\frac{\Delta OD}{\Delta t}$ is determined Thus, during the time Δt a change in optical density ΔOD occurs corresponding to a certain consumed volume of hemoglobin bound oxygen, a volume which is calculated in the following way

Knowing the oxygen binding capacity of hemoglobin, the hemoglobin concen

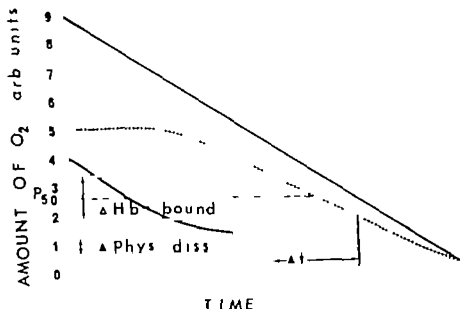


Fig. 4 Identical diagram to Fig. 2 ( ) lower part. The amounts of the partial fractions of oxygen consumed per unit time in the system in the vicinity of half-saturation (P_{50}) are illustrated. The dotted curve corresponds to an experimentally obtained recording (OD-curve).

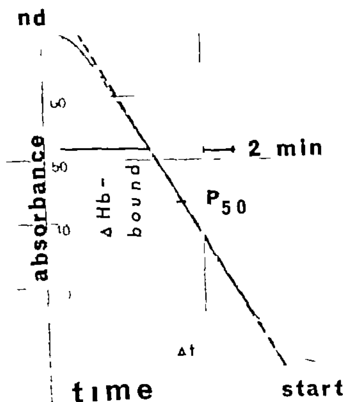


Fig. 5 An actual recording, OD-curve, from an experiment on isolated nerve cells. The fraction of the hemoglobin-bound O_2 pool as related to time in the vicinity of half-saturation is depicted. (Modified from Hultborn and Hydén 1974).

and the chamber volume the amount of hemoglobin bound oxygen (A) at complete saturation is known,

$A = V \times [HbO_2] \times 68\,000 \times 1.39$

where V = chamber volume

$[HbO_2]$ = hemoglobin concentration (M)

68,000 = nominal molecular weight of hemoglobin

1.39 = oxygen binding capacity of hemoglobin (ml O_2/g Hb)

This volume of oxygen also corresponds to the hemoglobin bound oxygen consumed by the cells at complete deoxygenation of the hemoglobin. The optical density of the incubate will change to a certain extent from full oxygenation to complete deoxygenation. This change is denoted ΔOD_{total} and it corresponds to the consumed volume of oxygen called A. Thus the volume of oxygen corresponding to Δ OD is $\frac{\Delta OD}{\Delta OD_{total}} \times A$

Since this volume of oxygen is consumed during the time Δ t, the rate of consumption of hemoglobin bound O_2 is obtained.

When using moderate ($<10^{-3}$M) or low concentrations of hemoglobin the physically dissolved fraction consumed during the time Δ t must be added. This is most easily done by determining beforehand in calibration experiments, the slope of the dissociation curve $\frac{\Delta z HbO_2}{\Delta P O_2}$ (z HbO_2 denotes saturation of the hemoglobin which is directly proportional to OD) by means of tonometry in the region of the P_{50} value. The reciprocal of the expression for this slope is proportional to $\frac{\Delta P O_2}{\Delta OD}$. From $\frac{\Delta P O_2}{\Delta OD} \times \frac{\Delta OD}{\Delta t}$ the expression $\frac{\Delta P O_2}{\Delta t}$ is thus achieved and the amount of physically dissolved oxygen that shall be added to the hemoglobin bound fraction is

$$\frac{\Delta P O_2}{\Delta t} \frac{\alpha}{760} V$$

where α = Bunsen's coefficient for gas solubility of the medium (vol. dissolved gas/vol. medium at a partial pressure of 760 mm Hg)

It is thus noted that no error is introduced when the hemoglobin concentration is low and thus the proportion of physically dissolved oxygen is increased since this fraction will be included in the calculations.

A correction of the volume of the incubation medium (V) might be necessary if large samples, displacing a significant fraction of the incubation medium are used.

Another approach of theoretical interest in the treatment of the OD-curve is the following. At the start of the experiment (zero time) the solution is equilibrated with air and the partial pressure of oxygen equals the volume per cent O_2 in air times the barometric pressure corrected for water vapour pressure. Since the hemoglobin is saturated at this pressure the oxygen bound to hemoglobin is known at zero time. Further from the solubility coefficient of oxygen the amount of physically dissolved oxygen at zero time is also known. Therefore and from the above considerations and Fig. 2 it is obvious that, whatever shape the oxygen dissociation curve has in the individual experiment it does not play any role with regard to the accuracy of calculations. Employment of these calculations also permits the determination of the oxyhemoglobin dissociation curves see p. 41

Hemoglobin solutions

Hemoglobin is preferentially prepared at the laboratory since commercially available preparations are frequently highly contaminated by methemoglobin and may contain other impurities harmful to cellular metabolism.

Preparation and storage.

Various types of preparatory procedures to obtain hemoglobin solutions exist and the one adopted in this work is not claimed to be the most accurate one although it has proved to be adequate for the purposes of the techniques described in this work.

Human whole blood, stored for two weeks at 4°C with acid-citrate-dextrose (ACD) + adenine was used. Initial volume for each batch was 300 ml The cells were rinsed with 0 15 M NaCl and centrifuged three times in the cold (1200 g for 10 min) whereupon they were rinsed with 0.17 M NaCl to decrease the cell volume. The cells were lysed by adding half the packed cell volume of redistilled water lysis being enhanced by shaking and freeze thawing. Cell debris were removed as a pellet after centrifugation at 50,000 g for one hour The clear hemolysate (~ 50 ml) was then placed on a Sephadex G-25 column (O 2.5 cm, height 40 cm) equilibrated with the buffer solution intended for use in the biological incubation experiments or with 0 15 M NaCl these solutions also being used to elute the column. The effluent was collected in fractions of 10 ml which were then adjusted to pH 7 4 Taking the molecular weight of hemoglobin as 68 000 the maximal hemoglobin concentration of these fractions was between 2 and 3×10^{-3}M The solutions were divided in 100 µl aliquots dispensed in small test tubes which are kept frozen at −80°C until used. At use the hemoglobin solution is diluted to the desired concentration in the incubation medium, often at relative volumes of 1:20 to 1:50 resulting in final hemoglobin concentrations in the range of 10^{-4}M

Characterization.

The sodium, potassium and cloride content of the hemoglobin solutions should be analysed to check if the potassium rich hemolysate was adequately changed in ionic composition on the passage through the Sephadex column.

Concentrations of the hemoglobin solutions were determined by the two-wavelength method. Since a gradual autoxidation to methemoglobin occurs on storage the amount of oxyhemoglobin was determined after aeration of the sample Absorption spectra from 600 to 400 nm were also recorded in oxygenized and reduced state for comparison with data in the literature In the study presented here a Zeiss double beam spectrophotometer was used for this purpose and extinction coefficients for hemoglobin and its derivatives were obtained from Assendelft (1970)

Crude hemoglobin-oxygen dissociation characteristics were also determined spectrophotometrically by use of a simple tonometric procedure The hemoglobin solution in the incubation medium, (~3 ml, concentration and composition as for an actual biological

experiment) is gassed with pure nitrogen for 20 min after which a spectrum from 600 to 500 nm is recorded (similar to that of a sample reduced by sodium dithionite) After subsequent bubbling, each 5 min, with gas mixtures (1 10 % O_2 & 98 90 % N_2 1 89 % O_2 & 98.11 % N_2 5 46 % O_2 & 94.54 % N_2 and air) the spectras of the solution from 600 to 500 nm are recorded From the data obtained an estimation of the half-saturation value of the hemoglobin can be derived as well as the steepness of the curve $\frac{\Delta z \, HbO_2}{\Delta PO_2}$ essential for the calculation of the oxygen consumption as discussed in the previous section. Typically P_{50} values obtained in these series of investigations were between 10 and 15 mm Hg and $\frac{\Delta z \, HbO_2}{\Delta PO_2}$ values were about 0 04 mm Hg^{-1} These dissociation characteristics should be determined whenever experimental conditions are changed (e.g. hemoglobin concentration, media constituents pH etc)

Determination of O_2-consumption in the range of 1 µl O_2/h

This section deals with the principles and procedures in determination of respiratory rates of cell suspensions and tissue homogenates, i.e. of particulate suspensions which will present specific problems when compared to the measurements of O_2-consumption of well-defined solid tissue samples treated in later sections.

Work with particulate suspensions introduces two major spectrophotometric artifacts, firstly there occurs a decrease of transmission of the incident light due to the presence of particles which diffract and absorb part of the light (c f Fog 1952 Wodick and Lubbers 1973) Since biological suspensions are extremely complicated in composition and structure no mathematical expressions for the degree of transmission can be used. Furthermore a change in diffraction and absorbing properties with time might be present. These complications are circumvented in the present system by the fact that no absolute absorbance values are required calculations are made purely on *changes* in absorbance of the *same* incubate (Δ OD) Furthermore a time-dependent change in diffraction is compensated for by use of a double beam spectrophotometer where the same biological suspension without indicator pigment is placed in the reference beam. The second spectrophotometric as well as respirometric artifact is due to particle sedimentation in the incubation chamber (cuvet) In conventional spectrophotometers this leads to a continuous passage of particles across the measuring light beam and to an increasing or decreasing concentration of particles in the volume traversed by the light beam. This can to a great extent be compensated for by a simple rearrangement of the measuring beam to a vertical direction so that the light passes through the cuvet in the same direction as the particles settle Further using short sedimentation distances the time of particle movements can be greatly decreased. The respirometric error introduced by particle sedimentation is due to aggregation and increased diffusion distances. In the type of cuvets described below this fact will not be of importance since almost a monolayer of particles will be present when sedimentation is finished. The measuring system described in this section is also equipped with an automatic cuvet changing device to increase measuring capacity

Incubation chambers (cuvets)

The incubation chambers used consist of commercially available fused 1 mm pathlength optical cuvets (visible light) with the outer dimensions 12 X 45 X 3 mm Suitable working volume of these cuvets is 200–300 µl The cuvet is sealed by the introduction of approximately 50 µl of liquid paraffin The liquid in the cuvet is held by capillary force so that the cuvets can be placed horizontally without risk of leakage After the experiment the cuvets should be cleaned as soon as possible The contents are thrown out by hand and the cuvets are then rinsed in running hot water for half a minute The cuvets are then immersed in a detergent solution for one day after which they are repeatedly rinsed in tap water reimmersed in absolute ethanol for at least one day and finally rinsed several times in distilled water and dried. Immersion of the cuvets is most suitably carried out in individual plastic vessels to avoid damage to the optical surfaces.

Analysing equipment

Basic unit A double beam recording spectrophotometer (Zeiss DMR 21) is used as the basic equipment. Monochromatic light is produced by a prism monochromator (M 4 QIII) with an automatically regulated slit width to give a constant reference signal which in turn can be altered by changing the degree of amplification For the special purpose of the technique described, the whole interior of the cell compartment was removed and replaced by components described below

Modifications. The alterations and new constructions mainly concern the cell compartment thus the rest of the instrument is only briefly interfered with The aim of the modifications was to combine the following four demands

a) to measure on horizontally positioned cuvets of the type described above
b) to produce a versatile piece of equipment for use with conventional cuvets
c) to have an automatic cuvet changer accommodating up to five samples and five references functioning both for horizontally positioned cuvets and conventional ones
d) to control temperature in both types of cuvets

The automatic cell changer is provided with two pairs of cuvet-wheels one of the pairs with holders for 1 mm pathlength cuvets in horizontal position *i.e.* for respirometry (Figs. 6 7 8) and the other with holders for conventional cuvets Both wheel-pairs are placed on a plain brass plate to which a synchronous motor is attached The brass plate is fastened to the bottom of the cell compartment Each cuvet-wheel has positions for five cuvets and each one can be in a measuring position from 5 to 60 sec varied by means of a timer within the control unit (Fig 9). The bottom brass plate is provided with a zig-zag channel for circulation of temperature regulated water

In the set-up for respirometry the light-beams passing through the cuvets have been changed to a vertical direction by an optical system including four plane surface coated mirrors in each beam. Two mirror-holders are mounted on the brass plate above the cuvet wheels by means of long knurled screws Each holder is provided with two mirrors inclined 45° to the horizontal plane the positions corresponding to two similar mirrors mounted in a groove in the brass plate Each light-beam is thus reflected four times within the cell compartment and the cuvet is placed above a circular hole (10 mm O) in the wheel between the third and the fourth mirror A stationary aperture (8 mm O) determines the dimension of the light beam in the plane of the cuvet while another diaphragm in front of the mirror holder prevents any direct light from passing to the light detector

To allow for an automatic cuvet changing function (Fig. 6) shaft pivots of the sample and reference wheels (gear wheel 100 cogs 100 mm O) are placed on the bottom-plate so that the wheels gear while one of them is driven by another gear wheel (40 cogs) connected to a synchronous motor (RSM 50/8 NG Berger Lahr West Germany) which has a speed of 5 r p.m. (cuvet wheels 2 r p m.). To make the wheels stop at appropriate positions there is a sensing lever fitting in excavations in the lower surface of the sample wheel corresponding to the positions of the circular holes in the wheel thereby influencing a microswitch which disconnects the motor via the control unit (Fig. 9). When in correct position recording is performed after a delay of 3 sec to allow the measuring system to stabilize Time of measurement can be set by use of a timer To avoid unnecessary wear of the potentiometer and monochromator slit motor these units

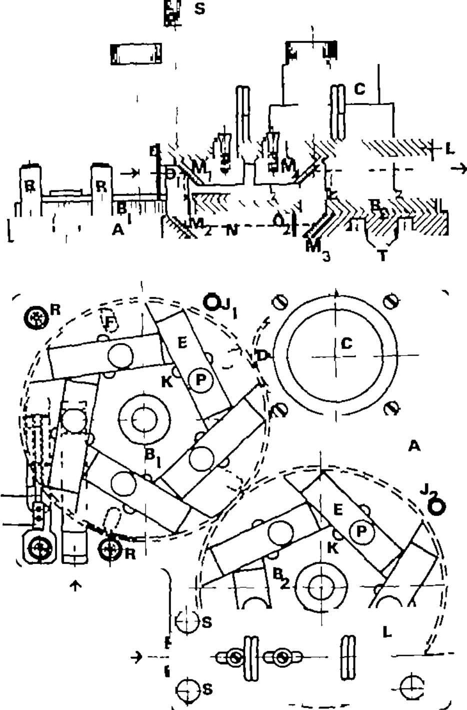


Fig. 6. Cuvet-changer as arranged fo spectrophoto-respirometry with horizontally positioned cells, lateral and vertical proje tions A base-plat B and B_2 sample and reference cuvet-wheels, C synchronous motor D driving gear wheel L cuvet in po ition I excav tions for G sensing lever H microswitch J_1 and J_2 inlet nd outlet f w for K spaces fo tweezer-grips, L mirror holder M_{1-4} mirrors, N groove for light beam O_1 and O_2 apert res, P holes in cuvet wheels for light passage; R, pillars for mirror holders, S knurled screws, T channel for temperat re regulated water (Modified from Hultborn *et al.* 1974)

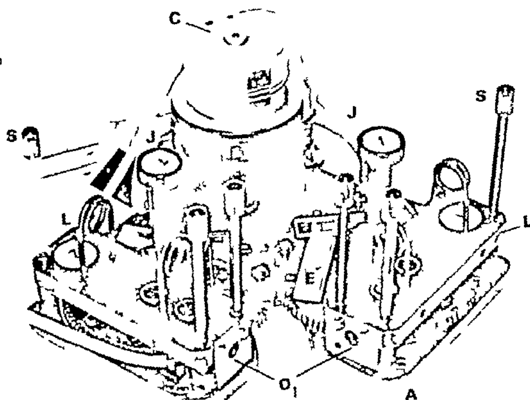


Fig. 7 The cuvet-change[r] completely assembled for spectrophoto- exp ometry Symbol in Fig 6 (From Hultborn *et al.* 1974).

are disconnected during cell-change The control unit operates at low voltage To allow an appropriate temperature regulation of the cuvets a channel (5 × 5 mm) is milled in the brass plate for circulation of water (Fig. 8). The heated brass plate transmits the heat to the cuvet wheels and the cuvets via the large contact area between the wheels and the brass plate

Working characteristics The accuracy of cuvet repositioning, *i.e.* cuvet wheel stopping, was examined for each cuvet position in the sample and reference wheel The angle accuracy is ±0.06 (1 SD) which is not critical especially since the light-beams will pass perpendicularly to the cuvet in any case and since the apertures are so arranged that the holes for the light beam in the cuvet wheels are not limiting

The extended pathway of the light beams (due to the mirror system) the reflections and the introduction of apertures decrease the amount of light passing through the cell compartment When there is no diaphragm in the beam the reduction of energy was found to be equivalent to that obtained when conventional microcells are used Since the instrument has a sufficient stability the decrease in energy does not cause any inconvenience in the type of measurments performed.

The efficiency of the thermostatically controlled system was tested at an ambient room

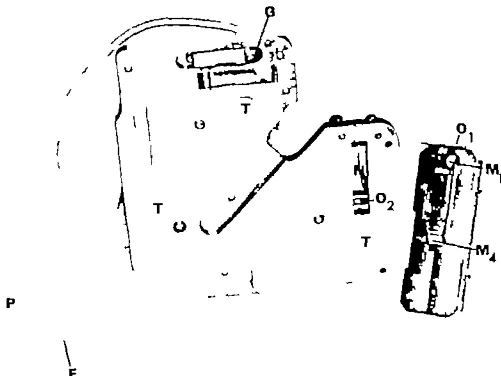


Fig. 8 From left to right the lower surfaces of sample cuvet-wheel for spectrophoto-respirometry the brass bottom-plate and one mirror holder Symbols as in Fig. 6 (From Hultborn *et al.* 1974)

...mperature of 20°C and the water was circulated at 37°C at a rate of 1.3 l/min. After one hour of equilibration without using the lid of the cell compartment there was a difference between inlet and outlet water of 0 1–0 2 C No difference in temperature (< 0 1°C) between sample and reference cuvets was found as studied with thermistors as well as encapsulated liquid crystals. The cuvets attained a temperature less than 0 5°C below that of the inlet water

Procedure

To a certain volume of the suspension which is to be analysed concerning its oxygen consumption is added a much smaller known volume of the concentrated hemoglobin solution. The final hemoglobin concentration is thus known. To ensure homogeneous distribution of biological material and pigment in the suspension as well as to complete the saturation of the hemoglobin with oxygen the solution is shaken and 200 to 300 µl of the suspension is transferred to the sample cuvets by means of a Pasteur pipette Liquid paraffin is placed on top of the suspension The cuvets should immediately be placed in horizontal position to allow sedimentation of particles and if a rapid respiratory rate is expected, it is advisable to place the cuvets on a cooled surface. The

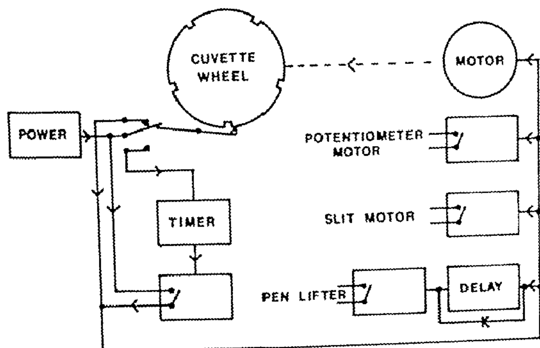


Fig. 9 Block diagram of the elect o-mechanical units of the cuvet-ch nge (From Hultborn et al 1974).

reference cuvets are filled with the same biological suspension as the sample cuvets, but do not contain hemoglobin and subsequently sealed as above The cuvets are then gently placed in their correct positions on the cuvet-wheels by use of a pair of tweezers. A spectrum from 600 nm to 500 nm is recorded to identify the oxyhemoglobin, whereupon the recording of the absorbance at 435 nm commences and continues until the absorbance change is finished. Another recording from 600 nm to 500 nm is performed to ensure that complete deoxygenation of the sample has occured.

Differential spectrophoto-respirometry

Use of a double beam spectrophotometer permits the recording of a difference spectrum (Hartmann 1937) of two "active cuvets" *i e* both sample and reference cuvets contain biological material incubated in the same concentration of hemoglobin. This procedure enables the identification of differences in respiratory rates of two suspensions which are identical except for the reactant or inhibitor the effect of which is to be studied Initially the contents of the two cuvets are oxygenated, so that the instrument indicates zero absorbance at any wavelength Thus any deflection in absorbance from zer measured at any wavelength except the isosbestic points indicates a difference of respiratory rates of the two suspensions (Fig. 10) Recording at a constant wavelength preferably 435 nm will in case of different respiratory rates result in a bell-shaped curve returning essentially to the initial absorbance value (zero), when the hemoglobin is completely reduced in both cuvets. In the sample (experimental) as well as in the



Fig. 10 A repeated wavelength-scanning differential-spectrophoto-respirometry of leucocytes incubated together with endotoxin in the sample cuvet to induce respiratory burst. Upper curves show the reaction of leucocytes from a normal individual, while the lower curve illustrates the absence of increased respiration of leucocytes from individuals suffering from chronic granulomatous disease. A wavelength scanning has been performed every 7th minute from 600 nm (left) to 400 nm (right). The difference between the reactions of normal and abnormal individuals can, with greatest accuracy be seen at 435 nm. (From Hultborn and Olling 1973).

reference (control) cuvets there will be an increase in absorbance with time. The total increase in absorbance from full oxygenation to complete deoxygenation is equal for both cuvets and is known from the hemoglobin concentration which also characterizes the shape of the absorbance shift curves, as described on p. 10. The bell-shaped curve is, in fact, the result of a subtraction of the absorbance shift curves of the two cuvets, and since the hemoglobin concentration is known they can roughly be reconstructed. However the amplitude of the deflection from the base line (zero) can be used as an indicator of the difference in the rates of hemoglobin reduction in the two cuvets. It must be emphasized that it is important to test that the substance whose effect on the respiratory rate is to be studied does not itself influence the oxygen affinity of the hemoglobin since this introduces erratic results for two reasons. Firstly the actual measurement of respiration would not occur at the same O_2 tension in the two cuvets and this might change the respiratory rate. Secondly even if the respiratory rates were the same the desaturation of the hemoglobin and thus the increase in absorbance would start earlier in the cuvet with the lowest O_2 affinity of the hemoglobin which would give rise to a deflection from the base-line.

Accuracy

An analysis of the slopes of the steepest parts of the absorbance shift curves (OD-curves) from ten experiments with a common yeast cell suspension yielded a remarkably good reproducibility ±2.2 % (1 SD) for the instrumental system together with the dissociation kinetics of the hemoglobin and pipetting errors (Hultborn *et al* 1974).

The differential-spectrophoto-respirometry should as yet be regarded as a semiquantitative yet sensitive method for detecting differences in respiratory activity of suspensions of common biological material

Final remarks.

The technique described in this section was developed using a Zeiss double beam spectrophotometer but it should be emphasized that a similar set-up can be made with other types of instruments, even single beam spectrophotometers can be used for the direct quantitative measurements. Another possibility would be to use the described cuvet changer as a separate unit connected to any spectrophotometer by the use of fiber optics. In this technique an instrument is utilized which is not exclusive for respirometry like most manometric equipments would be but can also be used in other spectrophotometric work. The method is easy to handle and not too sensitive to external influences, thereby making it suitable for routine use without special training

Determination of O_2-consumption between 10^{-2} and 10^{-4} $\mu l\ O_2/h$

The technique described in this section is applicable to well-defined small tissue samples down to isolated single cells in contrast to the technique described in the previous section. The measuring light-beam will in these cases not pass through a turbid solution, and the majority of spectrophotometric problems discussed above are thus not present.

The main outlines of the technique concern incubation of the tissue samples in minute chambers and the recording of the absorbance change of the hemoglobin solution in the chambers by means of a single-beam microscope spectrophotometer

Incubation chambers (cuvets).

General requirements

a) The volumes of the incubation chambers must be between 1 μl and 10^{-4} μl and be well defined

b) It must be possible to close the chamber so that no leakage of gas occurs.

c) The material in the walls of the chamber must be inert to oxygen so that no gas diffuses into the chamber from the walls. This is more imperative the smaller the incubation volumes are since the proportion of wall area to chamber volume is inverse to the volume

d) At least two opposite facets of the chamber must be transparent to visible light to allow the measuring beam to pass through

The depth of the chamber i.e. the optical pathlength of the chamber should not be small in relation to other dimensions of the chamber (e.g. in the case of cylindrical chambers the length of the cylinder should be in the range of or larger than the diameter) The reason for this requirement is twofold firstly to obtain a sufficient degree of absorbance without increasing the concentration of hemoglobin dramatically the optical pathlength should be as long as possible without increasing the volume of the chamber secondly the loading and closure of the chamber discussed later will be easier with the above mentioned geometrics.

f) The chambers should not be too delicate since they must be handled and cleaned after each experiment

It proves difficult to meet all these requirements adequately though the solutions described below fairly well fulfill the demands As for the material of which the chambers are produced, it turns out that glass and synthetic corundum, (crystallized Al_2O_3) are sufficiently inert to oxygen which is not the case for any plastic material. The surfaces of glass and corundum can furthermore be polished so that appropriate closure of the chamber is obtainable and the material is transparent to visible light.

Chambers used for O_2-uptake in the range of 10^{-2} $\mu l/h$. A suitable chamber volume for work in this range is approximately 1 μl One such type of 1 μl chamber can easily be produced by use of sintered carbide drills having a diameter of approximately 1 mm A bore is produced through a plane-parallel approximately 1 mm thick glass slide (conventional object slide) using a precision drilling machine To avoid cracks in the glass

surface the slide must be drilled from both sides, and to ensure that the drilled holes will meet perfectly a small excavation is first drilled in a metal-plate fixed in the stage of the drilling machine The glass slide is placed on this metal-plate and a bore is made to approximately half the thickness of it The slide is removed and a small sphere with a diameter similar to the drill is placed in the excavation of the metal plate The glass slide is turned upside down and placed so that the bore fits with the sphere during drilling. Slides with bores having cracks are discarded since volume determination of them would be difficult. The volume of the bore (chamber) is achieved from mechanical micrometer determination of the thickness of the slide and microscopic determination of the bore diameter To produce a bottom to the chamber a cover slip is fastened on to one side of the object slide with Nobecutan® (Bofors Nobel Pharma, Molndal Sweden) sucked in between the two glasses by capillary force As a cover of the chamber an object slide of a smaller size is firmly pressed on to the other glass. To be sure that the cover is tightly fitting, it is checked that interference fringes readily occur when juxtaposing the two object slides.

The described production of drilled chambers is suitable for multiple bore drilling in each glass-slide for use with an automatic cuvet-changing device described below The bores should then be placed in line with regular distances between each other conforming to the steplength of the cuvet changer Slides with six bores with distances of 3 mm have been used in this laboratory

Another type of chamber suitable for this range of work is made of synthetic corundum the sapphires being purchased of Seitz SA, Les Brenets, Switzerland The "hole jewels" used for this range of measurements have bore diameter of 1 00 mm outer diameter of 3.20 mm and thickness (*i.e.* height of the chamber) of 0.80 mm though other dimensions can be obtained as well as is indicated below The upper and lower surfaces of the sapphire are polished so that adhesion to an optically plane surface can be achieved by rubbing. The surfaces have also been controlled by interference microscopy Thus no adhesive is needed to produce a bottom. The jewel can simply be placed on an optically plane glass slide (cover glasses for hemacytometers, 0 6 mm thick, 30 × 20 mm) and charged with the sample As cover a smaller sized 5 × 5 mm glass slide of the same type is used.

Chambers used for O_2-uptake in the range 10^{-4}–10^{-6} µl/h. Chambers used for this range of respiratory rates suitably have volumes between 10^{-3} and 10^{-4} µl and due to these small volumes they can hardly be produced in the workshop of an ordinary laboratory Synthetic sapphire jewels with a precision bore in the centre (as above) can, however be purchased in various dimensions (Seitz SA Les Brenets Switzerland). Two dimensions of sapphire chambers have been used in this study having internal diameters of 0.250 and 0 040 mm, external diameters of 1 00 and 0.90 mm and thicknesses of 0 190 and 0 150 mm respectively The larger sized chamber having a volume of 1 3 × 10^{-3} µl (Fig. 11) is suited for measurements of respiratory rates in the range of 10^{-4} µl/h, while the smaller one is suited for the range of 10^{-6} µl/h. The surface finish of these chambers is the same as for those described in the paragraph above Since the sapphires are birefringent they can easily be visualized with polarized light which in the case of uncoloured jewels may be of importance (see below). The bottom and the cover of these sapphire chambers are arranged in a similar way to that described in the previous paragraph.



11. A sapphire jewel chamber with internal diameter and depth of 250 µm used f... ...nation of the respiratory rate of isolated Delters nerve cells. The cell, its nucleus and nucleolus ...learly visible within the chamber

In collaboration with Carl Zeiss (Oberkochen, West Germany) another type of "ultramicrochamber" has been produced in quartz glass A thin (70 µm) bored quartz glass is fused to another optically plane quartz slide The bore diameters vary from 60 to 100 µm, and the outer diameter of the glass is approximately 4 mm. The prime difference between the fused chambers and the sapphire chambers described above is that in the former type the chamber consists of one single unit in contrast to the separate sapphire and bottom in the latter type

The outer dimensions of the fused type of chamber are larger which make them easier to handle but the cleaning procedure for the sapphires is easier since they are both mechanically and chemically highly resistant By means of Pasteur pipettes (tip diameter slightly greater than that of the sapphire) they are sucked up and immersed in NaOH 0 2 M for a few hours in HCl, 0 ? M for one day in ethanol and finally in distilled water for two days. The fused chambers are more fragile and care should be taken not to expose them to alkaline solution or ultrasonic procedures. These chambers are more difficult to clean since they constitute a blind bore in contrast to the penetrating bore of the sapphires.

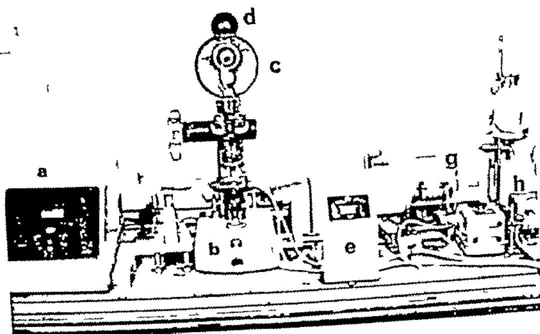


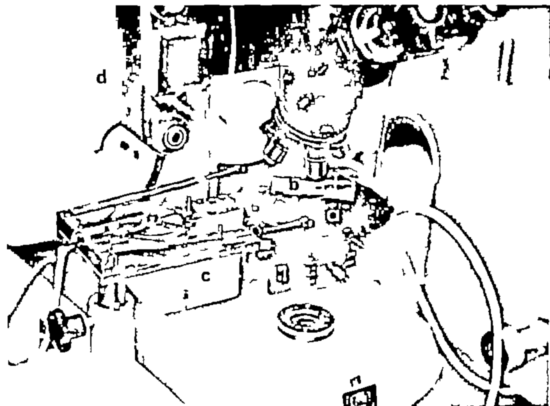
Fig. 1... Complete iew of the microspectroph tometric equipment used I rom left to right amplific (a) mic oscope (b) with photometer head ( ) and photomultiplier (d) monochromat   ( ) light source (f) with voltage stabilizer (g) and recorder (h)

Due to the restricted experience concerning the use of the Zeiss quartz-chambers they will not be further discussed in this work

General detecting equipment

A survey of the various components of the basic equipment used for microspectrophotometry in this type of work will be presented below It should be emphasized that most parts of the system are commercially available and that the whole equipment can be used for other purposes than oxygen consumption measurements such as ordinary mi roscopy microphotography cytophotometry etc without any alterations of the arrangements (Sorby 1867 Loofbourow 1950 Freed 1969 Pollister *et al* 1969) Photographs of the instrumentation are shown in Fig 1? 13 In this equipment Zeiss details have been compiled together with some components manufactured at the workshop n the laboratory

Light source Since the technique utilizes the visible range of electromagnetic adiation to which photomultiplier tubes are extremely sensitive and since the measu d   a are fairly la ge the light source need not to be of special high intensity Of great importance is work with single beam instruments in studies of time dependent reaction is to achieve a light source p oviding constant emission. Arc lamps of va ious types a e not suitable s the intensity and position of the a c frequently changes and thus the zero and I



3 Close-up view of the equipment. Temperature regulated object tage (a) with clamping tool T the left the cuvet-changing device ( ) nd camera (d) fastened on to an extra side-viewing ...

transmission settings can not be relied upon Filament (incandescent) lamps are therefore preferred, but the use of low-voltage sources makes it necessary to ensure that connections with the voltage supply are adequate (preferably soldered) Voltage stabilizers should be used in all cases. An auxiliary polychromatic illumination should also be provided for the purpose of centering and focusing an area of the chamber not occupied by the sample

Monochromator Various devices such as filters grating or prism monochromators can be used for spectral isolation In the present situation, where analysis of absorbance differences of hemoglobin compounds having rather sharp absorption characteristics, is to be performed it is advisable to use good instruments providing facilities for changing band widths and exact setting of wavelength Interference filters are therefore less suitable for this type of work By in reasing the band width less amplification of the cathode-current of the detecting photomultiplier is needed The diminished spectral resolution does, however result in a decrease in amplitude of the absorbance shift curve Furthermore it is of importance to have the visual field (object plane) uniformly illuminated with respect to wavelength This an be done by means of a prism- or grating monochromator with the exit slit as light source" i.e. according to Koh.er illuminated with respect to wavelength This can be done by means of a prism

Most commercial grating or prism monochromators fully meet the above mentioned demands. In the present study a Zeiss prism monochromator (M 4 QIII) was used.

Microscope and optics. Any mechanically stable microscope with facilities for flexible Kohler illumination can be used Side viewing tubes, one for ocular inspection of the specimen, should also be provided Above the object stage there must be ample space to mount a temperature regulated plate Centering and focussing facilities of the condenser (most suitably a low power objective) must be provided as well as the possibility of inserting various field iris diaphragms to obtain suitable dimensions of the measuring light-beam. Since measurements are made in visible light conventional optics can be used. Measured areas are always fairly large (>500 μm^2) and thus low-power objectives are used. The condenser should have a low numerical aperture since an increase in actual optical pathlength in relation to the nominal one otherwise will occur (Blout 1950) Achromatic lenses are fully sufficient for recording, though apochromatic ones are convenient when characterizing the spectrum of an incubate

In this study a Zeiss UVM II microscope was used with a 10 X N A 0 ? objective as condenser and as objective an apochromate 6.3 X N A 0 16 Field irises varied from 0.1 mm to 0.3 mm in diameter resulting in diameters of 15 μm to 45 μm in the object plane

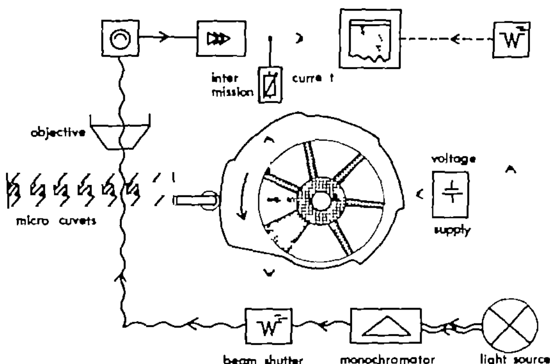
Photometer head and detector with amplifier The photometer head should provide facilities to inspect the image of the area to be measured Furthermore there should be different apertures, so that the area measured can be varied Electron multiplier tubes are excellent as detectors for measurements in visible light since they have a very high quantum efficiency The electronics should provide the possibility of varying the overall voltage of the photomultiplier tube as well as of changing the amplification of the anode-current. It is preferable to have the light modulated (*e.g.* by a beam-chopper) which allows the signals to be amplified using sharply tuned amplifiers with consequent rejection of much noise and diminished sensitivity to stray light The instrument should also be equipped with a galvanometer An RCA photomultiplier tube 1 P 28 was used in this work, generally at an overall voltage of approximately 580 V The amplifier light modulator and photometer head was of Zeiss MPM type

Recorder Since the absorbance change of the preparation studied is proportional to the relative amounts of the two hemoglobin compounds it is convenient to have a logarithmic transformation (from transmission to absorbance units) of the transmission signal fed from the amplifier Thus, the graphs obtained from the re order can be calculated upon directly In certain instruments this transformation" may be made at an earlier stage by holding the anode-current of the photomultiplier constant in which case the dynode voltage becomes a logarithmic function of the input light intensity The dynode voltage is then used as a measure of absorbance

The recorder should furthermore have an electromagnetically controlled hart-pen lifter easily made by the workshop of the laboratory for use with an autom ... changing device A Vitatron UR 405 Linear Logarithmic recorder was used in this study

Special equipment

Temperature regulation of the object Since cellular O_2-uptake i highly tempe... dependent it is essential to be able to control incubation tempe



14 Block diagram of the opto-electro-mechanical units in th microspectrophoto-respirometric s including the automatic cuvet changing device The program chart and cam me hanism re l in the centre. The dotted areas on the p ogram chart ar copper laminated and serve to open lose the circuit f auxiliary functions during cuvet change.

limits. Various electrically heated object stages are available but most of them do not permit easy handling of the preparation. Thus, it has proved most suitable to use a metal panel with channels inside connected with cold tap water and with a thermostatically precision regulated circulation waterbath. Temperature is calibrated by the use of melting substances within the incubation chambers (e.g. Eicosan $CH_3(CH_2)_{18}CH_3$ melting point 36 8°C which must be exactly determined for each batch purchased) The melting substance is placed in the chamber under similar conditions as the biological samples and during continuous microscopic visual inspection of the crystals, the temperature of the circulation waterbath is slowly increased until disintegration of the crystals occurs.

Automatic cuvet changing equipment To increase the measuring capacity a cuvet changing system has been developed as yet only used in determinations of O_2-uptake in the range of 10^{-2} µl/h (see p 25) The system is based on a cam mechanism with a disk cam ground to precise dimensions (six different radii with 3 mm steps) and a radial roller follower directly connected to the object stage The cam is driven by a synchronous motor at a speed of 0 5 r p.m. resulting in a measurement of all six chambers every two minutes. A beam-shutter prevents the photocathode from exposure to too intense a radiation during displacement (cuvet changes) Simultaneously a variable intermission current is fed to the recorder to avoid infinite absorbance Slightly before the

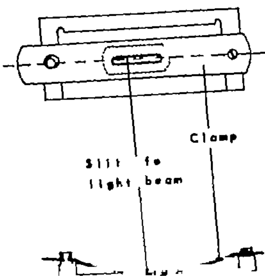


Fig. 15 The clamping tool used to press the cover glass on to the object slide with drilled chamber(s) as seen from above and the side.

beam-shutter and intermission current are operated the chart pen is raised electromagnetically The electronic functions are controlled mechanically by sliding contacts, following the rotating cam. For details see Fig. 14

Procedure

Loading of chambers drilled in object slides. Before introduction of the tissue sample a chilled chamber is filled with incubation medium including oxyhemoglobin The solution is introduced into the cavity by means of a pipette the tip diameter approximately 0.5 mm (pulled manually in a flame) so that the tip reaches the bottom of the chamber A rather large volume (~ 50 µl) of the medium is blown out so that the chamber is filled, and a large surplus amount of solution is situated on the glass surface around the chamber At this stage it should be checked that no air bubbles are trapped at the bottom of the chamber or on the walls, using phase contrast microscopy (low power objective) If a few bubbles are present they can easily be removed by means of a sharply pointed stainless steel needle The tissue sample can then be introduced into the chamber under a stereomicroscope The sample sediments rapidly to the bottom of the chamber and another check for air bubbles sticking to the sample should be made A smaller sized object slide is then applied as cover of the chamber by means of a pair of tweezers, and the surplus hemoglobin solution is pressed away If this moment is considered critical a tool described in the paragraph below may be used, Before the cover glass is firmly pressed on to the "chamber slide" it is once again checked that no air bubbles are present in the chamber The firm pressure is produced by the tool depicted in Fig. 15 The elongated slits in the tool serve for use with multiple-drilled glasses together with the cuvet-changing device described above The loading of the multiple-bore glasses is simular to that described above Care should however be taken to have the samples in the row of bores, all situated in the "upper" or "lower" regions of the chambers, so that the measuring beam will not be blocked by the sample in one or several chambers. This localization is achieved by tapping on the tilted compressed preparation which is

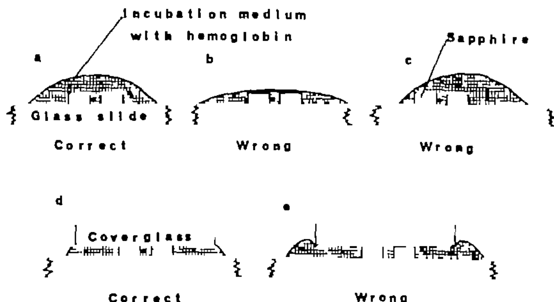


Fig. 16. Illustr tion of how the sapphire chamber should be located (a) in the drop of medium covering the whole sapphire (upper row). Too small amounts of medium (b) or an eccentric position of the sapphire in the drop (c) will cause disturbing turbulence of the liquid during the covering procedure resulting in escape of sample from the chamber. Too large a drop on the other hand will cause the cover glass to float on the surface of the liquid with sample loss as a consequence (e)

... on the object stage of the microscope. When using the cuvet-automaton it is ...ortant to orientate the preparation so that the row of bores exactly parallels the line ... back and forth movements of the object stage. Focusing and centering the specimen ... performed using polychromatic light. The field iris aperture is introduced and monochromatic light of 435 nm is allowed to enter the system. The amplification is then adjusted so that the galvanometer indicates an arbitrarily chosen low degree of absorbance (the recorder should cover the range of increase of absorption without changing settings during recording). Recording is then commenced and continued until a new stable ("reduced") level of absorption is achieved. The absorption spectrum of the solution in the chamber should occasionally (especially when starting a new experimental series) be plotted both before and after the experiment. The amplitude of the absorbance shift curve obtained (OD-curve) should also conform to the theoretically calculated value derived from the hemoglobin concentration.

Loading of sapphire chambers. Since the sapphires are not fixed to the underlying plane-parallel glass slide the filling procedure of these chambers will differ from that above. The incubation medium will in this case cover the whole jewel so that this is immersed in the hemoglobin containing drop. For the large sapphire bores this is easily done with a pipette as in the above paragraph. Fot the smaller sized bores (O ?50 μm and 40 μm) the chambers must be filled by means of delicate narrow tipped micropipettes. These are pulled in a classical electromagnetic puller intended for making glass microelectrodes. It is important to fill the bore first and not to immerse the whole jewel at once since the trapped air in the bore will be difficult to remove. The micropipette is charged from above by a syringe and is operated either by mouth or by a syringe with a

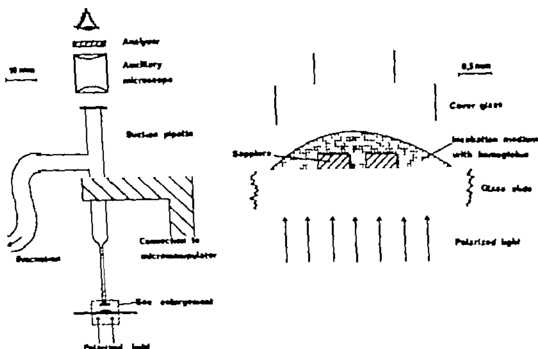


Fig. 17 Illustration of the equipment used to cover the sapphire chambers. The procedure minimizes liquid turbulence and consequently the risk f losing the biological material at this step. For the procedure see the text.

three-way stopcock. The filling is then carried out freehand under a stereomicroscope The way how the immersed sapphire should be located within the medium drop as well as some erroneous positions are depicted in Fig. 16 Since small volumes of aqueous solutions are handled it is of importance to avoid excessive evaporation. Hence the relative humidity of the laboratory should exceed 60 per cent and use of humidity chambers (moist Petri dishes) between operative steps is advised. The sample can now be introduced into the chamber through the surplus solution surrounding the sapphire a procedure which generally has to be done under stereomicroscope After spontaneous sedimentation of the sample to the bottom, which occurs rapidly the preparation is ready to be covered by a small sized glass slide Since the sample specimens are very small and the sapphires are not fixed to the glass underneath it is of importance not to cause too much turbulence of the liquid, when applying the cover a situation which may cause the sample to be sucked out of the chamber or even displace the whole sapphire A special tool was therefore constructed to minimize liquid turbulence As seen in Fig. 17 the cover glass is fastened by suction to a pipette connected to a manipulator movable in three directions. By an auxiliary microscope it is possible to see through the pipette along its a m, so that the cover glass can be exactly centered above the sapphire To visualize the small inconspicuous, birefringent sapphire it is illuminated from below by polarized light The pipette is slowly lowered so that the incubation medium makes contact with the cover glass and is then further lowered until the cover glass touches the sapphire The rubber tubing connected to the suction pipette is compressed manually and after a few seconds

/

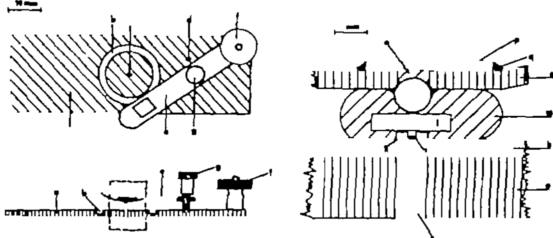


Fig. 18 Schematic drawing of the tool used to clamp the incubation chamber preparation. To the left top and lateral views of the whole tool are shown, and to the right an enlargement of the marked region is depicted. Plane-parallel brass plate (a) circular groove (b) central bore (c) excavation (d), clamp (e) bolt and knurled nut (f) knurled screw (g) plane-parallel glass slide (h) central bore in the sapphire, *i.e.* the incubation chamber (i) sapphire jewel (k) plane-parallel cover glass (l) immersion oil (m) glass sphere (n) adhesive or immersion oil with the same refraction index as the glass sphere (o) cover glass (p) adhesive (q) (From Hultborn and Hydén 1974).

the cover glass has detached from the pipette so that this can be raised. To avoid instability or even floating of the cover not too large a drop of incubation medium ould be used (Fig. 16) The preparation is inspected under a microscope to confirm that sample has not disappeared and that no air bubble has been introduced. The cover of e chamber should be firmly pressed on to the sapphire to prevent gas exchange with the nvironment. This clamping is fullfilled by a tool specially designed to attain accurately centric pressure (Fig. 18). The principle of the tool is to let the measuring light-beam, passing the chamber also pass through a glass sphere fused in a hole in the clamp thus ensuring a central pressure In spite of the relatively complicated optical system the image of the chamber is not much distorted, provided immersion oil is introduced in the system as indicated in Fig. 18 The whole preparation is now ready for transfer to the object stage of the measuring microscope Further procedures are similar to those described in the previous paragraph.

Addition of reactants in the course of an experiment

It is often an advantage to be able to study the metabolic response of a tissue to various reactants (*e.g.* substrates, hormones etc) using the same sample in order to avoid biological variation. A procedure has been worked out, for the "10^{-2} µl/h version where addition of a reactant can be made maintaining the same sample incubation chamber and hemoglobin concentration thus also minimizing technical errors. Drilled glass slides were used, but sapphires of the larger dimension can be used as well The procedures prior to the introduction of the reactant are identical to those described for these types of measurements above The recording should be allowed to continue until the increment in absorbance per unit time is strictly constant (between 75 and 50 % oxygen saturation)

The preparation is then removed from the heated object stage declamped and gently uncovered. The opened chamber including tissue sample is gently flushed with new hemoglobin-containing medium (~ 100 μl) now including the reactant. The flushing is performed under a stereomicroscope to ensure that no sample loss occurs. The preparation is then ready to be covered as described previously though for the case of drilled object slides a slightly smaller cover glass than that used the first time is applied to avoid contact with the crystallized material left at the border of that slide. The procedures are then similar to above and the recording is continued until complete deoxygenation occurs. The relative difference in "basal" and reactant induced respiratory activity can be directly read from the slopes of the linear part of the absorbance shift curves, and absolute respiratory rates are calculated as above. A prerequisite for this type of measurement is that the mixing (reoxygenation) *per se* does not influence the O_2-uptake of the tissue subjected to investigation. This is easily checked by running controls, *i.e.* without changing the composition of the medium for the second incubation.

General discussion

Similarities and dissimilarities to other microrespirometric methods with reference to O_2-tension.

It should be recognized that measurement of oxygen consumption is performed under drastically lower O_2 tension than is generally the case with the manometric procedures and in most cases with polarography This is due to the inherent principle of oxyhemoglobin dissociation in this system. To some degree the O_2 tension during "measurement" can be changed by adding ligands, such as 2,3 diphosphoglycerate or inositol hexaphosphate to the solution thereby increasing the half saturation value or by complete "stripping" of the pigment thus lowering the P_{50} value (Berman *et al* 1971) It has been shown that the hemoglobin solutions used in these experiments can easily attain an increased P_{50}-value (from 10–15 to 25 mm Hg) on addition of inositol hexaphosphate at hemoglobin equimolar concentrations (Hultborn unpublished)

It is important to consider the relatively low oxygen tension in the medium during measurements, with respect to oxygen gradients within the tissue sample or cell studied. Such gradients occur in solid tissue samples especially when respiratory rates are high and the samples are large This might cause an inhomogenous O_2-supply resulting in hypoxia in the centre of the tissue sample during the latter part of the experiment when the O_2 tension is getting lower Thus when estimating the respiratory rate of a sample under conditions, too low values as to the true respiratory capacity of the whole tissue are tained. Herlitz and Hultborn (1974) found in a study of the respiratory activity of solid samples of the corpus luteum from the rat, that the presence of the substrate succinate (inducing a high respiratory rate) resulted in a markedly asymptotic approach to the final "reduced level" of the OD-curve especially when large samples were used (~ 20 μg) but a much less pronounced asymptotic approach was noted in incubation experiments of such large samples when no substrate was present *i.e.* resulting in a fairly *low respiratory rate (Fig. 19) Small deviations from the theoretically expected final part* of the OD-curve was found in a study of the respiratory activity of isolated Deiters nerve cells, Fig. 5 (Hultborn and Hydén 1974) The deviations were similar to those obtained in an investigation on the O_2 uptake of brain homogenates (Hultborn and Jarlstedt 1974) but homogenates of corpus luteum presented extremly small deviations from the theoretically expected curve (Herlitz and Hultborn unpublished). These observations might reflect a different susceptibility to low O_2 tensions of different tissues.

A recent study by Starlinger and Lubbers (1973) on the respiration of mitochondria revealed that lowering of O_2 tensions down to 0.05 mm Hg did not affect oxygen uptake in contrast to previous reports where conventional O_2-electrodes were used (*e.g.* Lübbers 1968, Schuler and Kreuzer 1969) These latter studies indicate a decrease in respiratory rates in the range of a few mm Hg. The explanation for this discrepancy is the considerable inertia of the conventional O_2-electrodes (Starlinger and Lübbers 1973)

It might be claimed that the microrespirometric system presented here to a greater

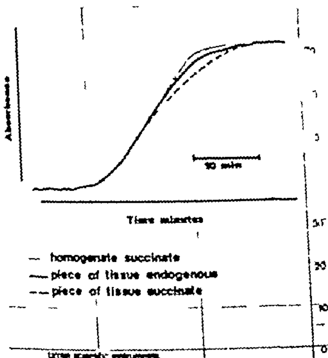


Fig. 19 OD-curves unde three different conditions. The middle curve is an actual recording, hile the two others ar reconstructed from other experiments. Observe the different approaches to the final reduced level indicating diff erent degrees of cellular hypoxia. (From Herlitz and H llborn 1974).

extent mimicks the physiological situation *in vivo* regarding oxygen tension than do the manometric procedures, almost invariably performed at atmospheric O_2 tension. This is of course only true when using small tissue samples or isolated cells not causing significant gas gradients.

In relation to oxygen tension and gradients within the medium in the chamber it should be noted that several studies have shown that hemoglobin promotes oxygen diffusion in liquids (e.g. Klug *et al.* 1956 Scholander 1960 Snell 1965 Wittenberg 1965) Further no differences of the degree of saturation of the hemoglobin at different distances from the sample have been noted in the incubation chambers in this investigation. A rapid equilibration of oxygen within the medium can therefore be assumed

Other pigments reversibly associated with oxygen can also be used as indicators of O_2-tension. For myoglobin however the P_{50} value is considerably lower than for hemoglobin, making it less suitable for routine microrespirometry However certain animal blood pigments (c f Altman and Dittmer 1971 Mill 1972) have high P_{50} values but the use of human hemoglobin is convenient, since it is easily obtained

Simultaneous to oxygen fixation in the tissues a continuous production of carbon dioxide takes place In the manometric procedures an alkaline solution absorbs the liberated CO_2 but this cannot be done in the polarographic or spectrophotometric procedures. The alkaline fixation of CO_2 in the manometric techniques is used to a... disturbance of the volumetric measurements. It can be calculated that in the chosen system adopted in the spectrophotometric technique using an adequately buffered system, no significant change in pH due to carbon dioxide will occur during the incubation. Furthermore determinations of O_2-uptake are always performed at or near half-saturation and the mere presence of hemoglobin potentiates the buffer

Oxygen consumption rates suitable for studies with the spectrophotometric method cover a wide range from 1 μl O_2/h to 10^{-6} μl O_2/h. The sensitivity can be varied on the one hand by changing the amount of oxygen available in the system *i.e.* by alteration of the chamber volume and hemoglobin concentration, and on the other hand by changing the amplitude of the OD-curve *i.e.* by variations of the hemoglobin concentration optical pathlength or wavelength for recording. For maximum sensitivity and resolution the volume of the chamber should be decreased as much as possible most efficiently done by reducing the diameter of the cylindrical chambers. Further a wavelength combining low transmission for hemoglobin with a pronounced relative difference in absorbance between the oxygenated and deoxygenated form should be chosen.

In general terms the resolution of the system treated here can be expressed as volume oxygen consumed per unit optical density change the resolution increasing with decreasing values of this ratio *i.e.* it is desirable to get a large change in OD for a small change of oxygen within the system. Due to the presence of physically dissolved oxygen, which is constant at a certain oxygen tension, an increase in hemoglobin concentration, leading to a proportional increase in amplitude of the OD-curve, will *not* result in a proportional increase in total oxygen content (chamber volume and optical pathlength kept constant) Thus the volume oxygen per unit OD change (dotted curves in Fig. 20) will decrease as the hemoglobin concentration is raised implying an increased resolution. These considerations suggest the use of very high hemoglobin concentrations, which would result in greatly prolonged times for completion of the OD-curves, due to the ... amount of oxygen in the system. It was therefore calculated how much is ... in resolution on raising the hemogobin concentration and in Fig. 20 it should be ...rved that hemoglobin concentrations (tetrameric) above 10^{-4} M do not add much to ...olution. In Fig. 20 the ratio has been plotted against hemoglobin concentration at two ...ifferent oxygen tensions of the system, namely at 150 mm Hg (atmosphere) and at 50 mm Hg, the hemoglobin being assumed to be fully saturated with oxygen at both these tensions, which is approximately "true" for the hemoglobin solutions used in this investigation. The ratio has lower values at the lower oxygen tension since the proportion of hemoglobin-bound O_2 to physically dissolved O_2 has increased due to the decrease of the physically dissolved pool. The ratio will not decrease much more at further lowering of the O_2 tension since the oxyhemoglobin will then progressively dissociate.

In this context it should be emphasized that completion of the OD-curve in each experiment is not necessary since the ratio of the volume O_2 per unit change in OD is known as discussed above Therefore only a small section of the linear part of the OD-curve has to be recorded, a procedure partly applied by Herlitz and Hultborn (1973) in mixing experiments. Such a procedure significantly increases measuring capacity since time of measurement can be kept very short, especially if the preparation is flushed with a gas mixture having an oxygen tension of 20–50 mm Hg before closing the chamber thereby reducing the amount of physically dissolved oxygen.

The resolution was above defined as the ratio of the volume consumed oxygen per unit change in optical density However various detecting instruments have different capacities to amplify a certain OD-change to make it measurable at the data output level. The signal to noise ratio of the detecting equipment generally does not play any role since the time

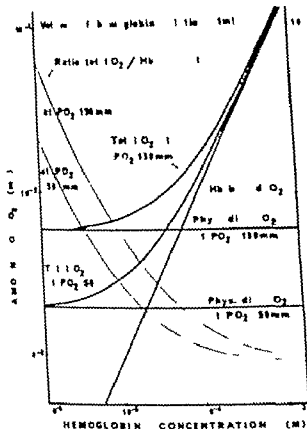


Fig. 6 Illustration of the amount of hemoglobin-bound and physically dissolved oxygen and the sum of these with changing hemoglobin concentration. The situation is illustrated for two different O_2 tensions, the hemoglobin being assumed to be fully saturated with oxygen at both. The expression for the resolution of the method, the ratio of the volume O_2 per unit change in optical density, is illustrated by the dotted curves. In the figure OD is replaced by Hb-concentration since they are directly proportional to each other. It is seen that not much is gained in resolution on increasing the hemoglobin concentration above 10^{-4} M. A slight decrease in amount of physically dissolved oxygen with increasing hemoglobin concentration is disregarded. For further discussion see the text.

constant of the kinetic reaction of the object is so large that adequate damping can be used. For single beam instruments it is necessary to test the long time stability so that no drifts in signal occurs

Another way of increasing resolution of the system and thereby the sensitivity is to use a reflecting microscope and thus have the measuring light-beam reflected at the bottom of the chamber. The optical pathlength is then doubled and an increased change in optical density per volume oxygen consumed is achieved

The reproducibility of the system as used in this investigation is dependent on the constancy of the various parameters included, such as accurately determined chamber volumes and hemoglobin concentrations. The constancy of the oxyhemoglobin kinetics is of importance both for the true respiratory rate of the biological material studied (a drastic decrease in half-saturation value could decrease respiratory rate) and for the calculations on the obtained OD-curve. Hultborn *et al* (1974) using the 1 µl O /h version found from an analysis of ten experiments with a common yeast cell suspension a reproducibility of ± % (1 SD) for the instrumental system together with the dissociation kinetics of the hemoglobin. Herlitz and Hultborn (1974) using the 10^{-3} µl O_2/h modification found in a study of respiration of corpus luteum slices 1.06 ± 0.14 µl O /mg dry weight × h (mean ± SD n = 13). No oxygen gradient leading to significant central anoxia of the sample were present to add to the variation however a considerable error of weight measurement was included in the biological variation. In conclusion it seems that the reproducibility of

spectrophotometric system is the same as or even better than the micromanometric techniques (Herlitz and Hultborn 1974)

Other considerations specific for the spectrophoto-respirometric system.

The system investigated contains a protein as indicator of oxygen tension The inherent presence of a protein in the system must be considered as a potential risk of interference with the metabolic events subject to study A direct "toxicity" to the tissue must be ruled out as well as interference with substrates hormones etc. present in the incubation medium. Hultborn (1972) made a dose response study of yeast cell respiration varying *the hemoglobin concentration from* 5×10^{-6} *to* 1×10^{-3} *M without finding any* significant covariation in respiratory rates. Bârzu and Borza (1967) did not find any relation between hemoglobin concentration and the oxidative activity of isolated mitochondria. Further Herlitz and Hultborn (unpublished) performed standard diver respirometry on samples of corpus luteum in the presence of 5×10^{-5} M oxyhemoglobin in the medium of the diver without finding any difference in respiratory rates from control divers without hemoglobin. These data substantiate the suggestion that hemoglobin generally does not influence on tissue metabolism. In the diver experiments mentioned above succinate was used as substrate and the action of this was obviously not interfered with by the protein present.

Since temperature is a parameter on which metabolic activity is highly dependent it was investigated whether the measuring beam, due to its partial absorption in the medium, could raise the temperature of the sample. The energy flux through the object was measured by a calibrated photomultiplier tube the cathode placed near the object plane without any object in the path of the light beam. Assuming complete absorption of the energy in the medium calculation of the "heat" dissipation through the material surrounding the chamber discloses that a completely insignificant increase in temperature occurs in the chambers due to energy absorption from the incident light beam.

Since it is of prime importance that no gas exchange occurs between the chamber and the environment it was tested that a hemoglobin solution deoxygenated by nitrogen flushing was not reoxygenated within the closed chamber It was also tested that an oxyhemoglobin solution in a closed chamber was not transformed to carboxyhemoglobin when the whole preparation was situated in a carbon monoxide environment

Facility and versatility

In a study comparing the classical "standard-diver" technique and the spectrophotometric one (Herlitz and Hultborn 1974) it was stated that the whole procedure was considerably more convenient using the latter method By use of automatic cuvet changers the capacity of measurements is obviously increased. The automatic recording involved in spectrophotometry is also advantageous over the discontinuous manual determinations most oftenly used in micromanometry Without any changes of equipment except for the chambers respiration rates from 10^{-2} to 10^{-4} $\mu l\ O_2/h$ can be measured

The microspectrophotometric equipment used for this type of investigation can without any alterations be used for other purposes than microrespirometry such as ordinary microscopy microphotography cytophotometry etc

Applications.

The microrespirometric system described in this work has been applied to various tissues under different conditions. Below a brief review of these studies will be given.

The "1 µl O_2/h" version suitable for measurements on cell suspensions or tissue homogenates was used by Hultborn (1972) in a study of yeast cell respiration at various concentrations of hemoglobin in the medium (see above). Hultborn and Olling (1973) investigated the metabolic properties of leucocyte enriched fractions from normal blood-donors and from individuals suffering from chronic granulomatous disease (CGD). Normally granulocytes exhibit a marked increase in respiratory activity "respiratory burst" on stimulation to phagocytosis, but cells from patients suffering from CGD lack this "burst" which was easily demonstrated by use of the differential-spectrophotorespirometry (Fig. 10). Further the respiratory rates of tissue homogenates from brain and liver (Hultborn and Jarlstedt 1974) and from the corpus luteum of the rat (Herlitz and Hultborn unpublished) were investigated.

Investigations utilizing the microscope spectrophotometer equipment include a study of the respiratory activity of small samples of well defined corpus luteum from the rat (Herlitz and Hultborn 1974). The influence of different substrates on the O_2-uptake was studied and the results were compared with those obtained in experiments using "classical standard-divers". Further (Herlitz and Hultborn 1973) reported succesful addition of substrates in the course of an experiment as well as the use of the automatic cuvet changer. Hultborn and Hydén (1974) studied the metabolic response to increased potassium concentration of isolated Deiters' nerve cells (Figs. 5, 11) where a profound increase in O_2-uptake in the presence of 50 mM KCl as compared to 4 mM KCl was found. The oxygen consumption of small numbers of yeast cells (5–20 cells) were determined by Hultborn (1972) who used an early version of the "ultrasmall" incubation chambers suitable for oxygen uptakes in the range of 10^{-4} µl/h. The sapphire jewel chambers suitable for this range of work have been tested using yeast cells (Hultborn unpublished).

Investigations are in progress (Hultborn and Rockert unpublished) where capillaries (internal diameter 0.4 mm, length 30 mm) are filled with solutions of brain homogenate at a pressure of 6 atmospheres (air) the homogenate suspension being equilibrated with air at this pressure beforehand. As expected, the time elapsed before the start of the dissociation of the oxyhemoglobin was prolonged. Thus indicates the possibility to use this system for determination of oxygen dissociation curves by an approach related to that published by Longmuir and Chow (1970), though in their study oxygen tension is traced instead of hemoglobin saturation.

Assessment of cellular and tissue metabolism has not been commonly used for functional categorization of oncologic and metabolic diseases (c.f. Warburg 1962). This situation is due to the hitherto wellknown complexity and difficulty of such analyses. The relative simplicity of the method presented in this work may however open new possibilities for such functional studies in combination with cytological and histological examinations on material obtained by different types of biopsies. This would introduce a new dimension in classification of neoplastic diseases, and furthermore it permits testing of potential therapeutics *in vitro*.

Summary

1 A method is presented suitable for measurements of O_2-uptakes between 1 μl/h to 10 ^{6} μl/h.

2. The sample subject to study is incubated in a closed chamber (cuvet) in a medium containing oxyhemoglobin. The gradual deoxygenation of the hemoglobin, due to the *sample respiration, is recorded spectrophotometrically at 435 nm.*

3 *The theoretical* background of this system as well as an analysis of the resolution of the system are presented.

4 The relatively low oxygen tension at which measurement of respiration is performed using this technique is discussed in relation to manometric measuring systems and to the situation *in vivo*

5 A dual-beam spectrophotometer was adapted for assessment of oxygen consumption of cell suspensions and homogenates in the range of 1 μl O_2/h. To increase measuring capacity a cuvet-changing device was developed.

6 For determinations of respiratory rates of solid tissue samples or isolated cells a single-beam microscope spectrophotometer was used. Incubation of samples was made in glass or sapphire cuvets of minute dimensions. The sensitivity was brought to the range of 10^{-6} μl O_2/h.

7 The versatility facility and accuracy of this method were compared with those of a micromanometric procedure It was found that the microspectrophotometric respiro-...y was more easily operated than was the diver procedure The accuracy was as high as or even better than that for micromanometry

Brief notes on investigations applying the spectrophoto-respirometric procedure to various biological materials, such as blood cells, brain and ovarian tissues are given

Acknowledgements

I wish to express my deep gratitude to my chief Professor Holger Hyden for his never failing interest in my work. His spirit and active support have been of the greatest importance for this work.

I am also very grateful to Hans Herlitz, Sune Larsson, Sante Olling and Lennart Pilhage for stimulating collaboration and valuable discussions.

I am greatly indebted to Lars Hamberger for his most active support and interest throughout this investigation and to Jan Jarlstedt for his great generosity and interest.

I also wish to thank Yngve Källström and Sten Rosberg for much help and discussions, Eva Åwall, Kerstin Lundmark and Clarence Hall for patient assistance, Gull Grönstedt and Hans Wilson for skillful secreterial work.

My sincere thanks are also due to Graham McLean for correcting the language.

This work was supported by grants from the Swedish Medical Research Council (Project no B74-13X-86-10B), the Medical Faculty, University of Goteborg, Svenska Sällskapet för Medicinsk Forskning, Assar Gabrielsson's fund for clinical cancer research, Helge Axson Johnsons Foundation, Wilhelm and Martina Lundgrens Foundation and Stiftung Volkswagenwerk.

References

ADAIR G.S The hemoglobin system VI. The oxygen dissociation curve of hemoglobin. *J Biol. Chem.* 1925 *63* 529–545

ALTMAN P L. and D S DITTMER, Respiration and circulation. *Biological Handbooks.* Fed. Amer Soc. Exp Biol. 1971

ARSONVAL, A. Photographie des spectres d absorption de l'hemoglobine et de son emploi en physiologie et en médecine légale. *Arch. Physiol.* 1890 *2* 340–346

ARVANITAKI, A. and N CHALAZONITIS Réactions bioélectriques neuroniques à la photoactivation spécifique d'une héme-protéine et d une carotène-protéine *Arch. Sci. Physiol.* 1949 *3* 27–44

ASSENDELFT O W v *Spectrophotometry of haemoglobin derivatives.* Royal Van Gorcum Assen. 1970

BÂRZU O and V BORZA Spectrophotometric method for assay of mitochondrial oxygen uptake using oxyhemoglobin as indicator and oxygen donor I. *Anal Biochem.* 1967 *21* 344–357

BÂRZU O L. MURESAN and G BENGA Spectrophotometric method for assay of mitochondrial oxygen uptake IV *Anal. Biochem.* 1972 *46.* 374–387

BARZU O L. MURESAN and C. TARMURE, Spectrophotometric method for assay of mitochondrial oxygen uptake II. *Anal. Biochem.* 1968 *24* 249–258.

BARZU O and M SATRE, Determination of oxygen affinity of respiratory systems using oxyhemoglobin as oxygen donor *Anal. Biochem.* 1970 *36* 428–433

BENESCH, R. and R. E BENESCH, The effect of organic phosphates from the human erythrocyte on the allosteric properties of hemoglobin. *Biochem Biophys. Res. Commun.* 1967 *26* 16?–167

BENESCH, R. R E. BENESCH and C I YU Reciprocal binding of oxygen and diphosphoglycerate by human hemoglobin *Proc Nat Acad Sci.* 1968 *59* 526–532.

BENESCH R. G MACDUFF and R. E. BENESCH, Determination of oxygen equilibria with a versatile new tonometer *Anal. Biochem.* 1965 *11* 81–87

BERMAN M R. BENESCH and R E. BENESCH, The removal of organic phosphates from hemoglobin. *Arch. Biochem. Biophys.* 1971 *145* 236–239

BLOUT E. R. C R. BIRD and D S GREY Infra-red microspectroscopy *J Opt. Soc. Amer* 1950 *40* 304–313

BOHR, C K. A HASSELBALCH and A. KROGH Ueber einen in biologischer Beziehung wichtigen Einfluss den die Kohlensäurespannung des *Blutes* auf dessen Sauerstoffbindung übt *Skand. Arch. Physiol.* 1904 *16* 40 –41

CHALAZONITIS N Intracellular pO_2 control on excitability and synaptic activability in *Aplysia* and *Helix* identifiable giant neurons *Ann. N Y Acad Sci.* 1968. *147* 419–459

CHANCE B Reaction of oxygen with the respiratory chain in cells and tissues. *J Gen Physiol* 1965 *49* No *1* part ? 163–188

CHANUTIN A and R. R CURNISH Effect of organic and inorganic phosphates on the oxygen equilibrium of human erythrocytes. *Arch. Biochem. Biophys.* 1967 *121* 96–102.

COLMAN C. and L S LONGMUIR, A new method for registration of oxyhemoglobin dissociation curves. *J Appl Physiol* 1963 *18* 420–4 3

DENNIG A., Spectralanalytis he Messungen der Sauerstoffzehrung der Gewebe in gesunden und kranken Zuständen *Ztschr Biol.* 1883 *19* 483–500

FOG J Turbidity in photometry *The Analyst* 1952. 77 454–460

FREED J J Microspectrophotometry in the ultraviolet spectrum. *Physical Techniques in Biological Research* Acad Press, New York & London. 1969 *III C* 95–167

GAMGEE, A., On the absorption of the extreme violet and ultra-violet rays of the spectrum by haemoglobin, its compounds and certain of its derivatives. *Ztschr Biol.* 1897 *34* 505–528.

GIBSON Q H. and R. D GRAY The reaction of inositol hexaphosphate with hemoglobin. *Biochem. Biophys. Res Commun.* 1970 *41* 415–420.

HARTMANN H., Über die Grundlagen der Differenzphotometrie und ihre Anwendung zur Bestimmung geringer Kohlenoxydmengen im Blut *Ergeb Physiol.* 1937 *39.* 413–449

HERLITZ H. and R. HULTBORN Advances in a spectrophotometric procedure for measurements of oxygen consumption on microscale. *Acta physiol scand.* 1973 Suppl. *396.* 116

HERLITZ, H. and R HULTBORN A microspectrophotometric technique for determination of respiration in comparison to the Cartesian diver method. – Respiratory activity of rat corpus luteum cells with reference to substrate *Acta physiol. scand.* 1974 In press.

HEYROVSKY J Process at mercury dropping cathode *Trans. Faraday Soc.* 1923–24 *19* 785–792.

HILL, A. V., The possible effects of the aggregation of the molecules of haemoglobin on its dissociation curves. *J Physiol.* 1910 *40* IV–VII

HOLTER, H. and E. ZEUTHEN Manometric techniques for single cells. *Physical Techniques in Biological Research.* Acad. Press, New York & London. 1966. *III A* 251–317

HOPPE, F., Ueber das Verhalten des Blutfarbstoffes im Spectrum des Sonnenlichte *Arch. Pathol. Anat* 1862, *23* 446–449

HULTBORN R., A sensitive method for measuring oxygen consumption *Anal. Bioche* 1972, *47* 442–450

HULTBORN R. and H HYDÉN Microspectrophotometric determination of nerve cel respiration at high potassium concentration. *Exp Cell Res.* submitted for publicatio

HULTBORN R. and J JARLSTEDT Effect of ethanol on the oxygen consumption of cerebral cortex, cerebellar cortex and liver homogenates. *J Neuropath. Exp. Neur* 1974 In press.

HULTBORN R. S LARSSON and L. PILHAGE, An automatic cuvet chan spectrophotometric oxygen consumption measurements and for conventional photometry *Zeiss Informationen* accepted for publication. 1974

HULTBORN R. and S OLLING Studies on leucocyte function by measuring and nitrobiu tetrazolium reduction by simplified methods. *Scand J Clin* 1973 *32.* 297–304

KAHN R. H. Eine Method der Spektroskopie des Hämoglobins ... *Pflügers Arch.* 192_ *195* 361–365

KILMARTIN J V and L. ROSSI BERNARDI, Interaction of hemoglob ions, carbon dioxid and organic phosphates. *Physiol. Rev* 1973 *53*

KLUG, A. F KREUZER and F J W ROUGHTON The diffu concentrated haemoglobin solutions. *H* ... *Physiol. Acta* 1956 *14*

KOCH M. Spektralanalytische Untersuchungen der Gewebsatmung am Menschen. *Ztschr Klin. Med* 1928 *109* 403–413
LESSLER M A. and G P BRIERLY Oxygen electrode measurements in biochemical analysis. *Methods of Biochemical Analysis* 1969 *17* 1–29
LINDERSTRØM LANG K Principle of the Cartesian diver applied to gasometric technique. *Nature* 1937 *140*. 108
LONGMUIR I S and J CHOW Rapid method for determining effect of agents on oxyhemoglobin dissociation curves. *J Appl. Physiol.* 1970 *28*. 343–345
LOOFBOUROW J R., Microspectroscopy *J Opt. Soc Amer* 1950 *40* 317–325
LÜBBERS D W Intercapillärer O_2 Transport und intracelluläre Sauerstoffkoncentration. *Biochemie des Sauerstoffs*. 19 Colloq Gesellsch. Biol. Chem. 1968 pp 67–92.
LÜBBERS D W The meaning of the tissue oxygen distribution curve and its measurement by means of Pt-electrodes. *Progr Resp Res* 1969 *3* 112–123
MACMUNN C. A. *The spectroscope in medicine* London, Churchill 1880
MEYER, E. and A. REINHOLD Untersuchungen über die Gewebsatmung am Lebenden. *Klin Wschr* 1926 *5* 1692–1696
MILL, P J., *Respiration in the invertebrates*. MacMillan, St. Martin s Press. 1972.
NICOLAI L. Über Sichtbarmachung, Verlauf und chemische Kinetik der Oxyhämoglobinreduktion im lebenden Gewebe besonders in der menschlichen Haut *Pflügers Arch.* 1932. *229* 372–384
NICOLAI L. Fortgesetzte Untersuchungen über den Verlauf der Oxyhämoglobinreduktion in der menschlichen Haut *Pflügers Arch* 1932 *230* 238–245
NILSSON N J Oximetry *Physiol. Rev* 1960 *40* 1–26
OMAN S and M BRZIN The magnetic diver microgasometer *Anal. Biochem.* 1972. *45* 112–127
PAYNE, J P and D W HILL, *A symposium on oxygen measurements in blood and es and their significance* J & A. Churchill Ltd 1966
ISTER, A W H SWIFT and E. RASCH, Microphotometry with visible light. *Physical Techniques in Biological Research.* Acad. Press, New York & London. 1969 *VIII C* 201–251
RØRTH M *Hemoglobin interactions and red cell metabolism* Thesis. Copenhagen, 1973
RØRTH, M and P ASTRUP *Oxygen affinity of hemoglobin and red cell acid base status*. Proc. of the Alfred Benzon Symp IV Copenhagen Munksgaard. 1972.
ROUGHTON F J W The accurate gasometric determination of the oxygen-hemoglobin equilibrium in dilute solutions of sheep hemoglobin *Clin. Chem.* 1963 *9* 682–709
SCHOLANDER P F Oxygen transport through hemoglobin solutions. *Science* 1960 *131* 585–590
SCHULER, R. and F KREUZER Properties and performance of membrane-covered rapid polarographic oxygen catheter electrodes for continuous oxygen recording *in vivo Progr Resp Res.* 1969 *3* 64–77
SICK, H. and K. GERSONDE Method for continuous registration of O_2 binding curves of hemoproteins by means of a diffusion chamber *Anal. Biochem.* 1969 *32*. 362–376
SNELL, F M. Facilitated transport of oxygen through solutions of hemoglobin. *J Theoret. Biol.* 1965 *8* 469–479
SORBY H. C., On a definite method of qualitative analysis of animal and vegetable colouring-matters by means of the spectrum microscope. *Proc. Roy Soc.* 1867 *15* 433–455

SORET M. J L., Recherches sur l'absorption des rayons ultra-violets par diverses substances. *Arch. Sci. Phys. Nat.* 1878 *61* 322–359

SORET M. J L. Recherches sur l'absorption des rayons ultra-violets par diverses substances. *Arch. Sci. Phys. Nat* 1883 *10.* 429–494

STARLINGER, H. and D W LÜBBERS Polarographic measurements of the oxygen pressure performed simultaneously with optical measurements of the redox state of the respiratory chain in suspensions of mitochondria under steady-state conditions at low oxygen tensions. *Pflügers Arch.* 1973 *341* 15–22

STOKES, G G On the reduction and oxidation of the colouring matter of the blood. *Proc. Roy Soc.* 1864 *13* 355–364

UMBREIT W W., R H BURRIS and J F STAUFFER *Manometric and Biochemical Techniques.* Burgess Publ. Co 1972

VAN SLYKE, D. D and J M NEILL, The determination of gases in blood and other solutions by vacuum extraction and manometric measurement. *J Biol. Chem.* 1924 *61* 523–573

VIERORDT K. *Die Anwendung des Spectralapparates ur Photometrie der Absorptions spectren und zur quantitativen chemischen Analyse.* Laupp schen Buchhandlung, Tubingen. 1873

VIERORDT K. Physiologische Spektralanalysen VIII Das Hämoglobulinspektrum am lebenden Menschen. *Ztschr Biol.* 1875 *11* 195–197

VIERORDT K., Physiologische Spektralanalysen. IX Die Sauerstoffzehrung der lebenden Gewebe *Ztschr Biol.* 1878. *14* 422–448

WARBURG O *Über den Stoffwechsel der Tumoren.* Verlag v Julius Springer Berlin. 1926.

WARBURG, O *New methods of cell physiology* Interscience Publ. New York 1962.

WITTENBERG J B Myoglobin-facilitated diffusion of oxygen. *J Gen. Physiol.* 1965 *49* No. *1* part 2 57–74

WODICK, R and D W LÜBBERS Methoden zur Bestimmung des Lichtweges bei der Photometrie trüber Lösungen oder Gewebe mit durchfallendem oder reflektiertem Licht. *Pflügers Arch.* 1973 *342* 29–40

WODICK, R. and D W LÜBBERS, Ein neues Verfahren zur Bestimmung des Oxygenierungsgrades von Hämoglobinspektren bei inhomogenen Lichtwegen, erläutert an der Analyse von Spektren der menschlichen Haut. *Pflügers Arch.* 1973 *342* 41–60

ACTA PHYSIOLOGICA SCANDINAVICA,
SUPPLEMENTUM 405

The effect of quantitated training on the capacity for short and prolonged work

By
Lars-Olof Nordesjö

Stockholm 1974

ACTA PHYSIOLOGICA SCANDINAVICA,
SUPPLEMENTUM 405

From the Military Medical Examination Center (MMUC) and the Department of Clinical Physiology Karolinska sjukhuset, Stockholm, Sweden.

The effect of quantitated training on the capacity for short and prolonged work

By
Lars-Olof Nordesjö

Stockholm 1974

CONTENTS

	ABSTRACT	3
	LIST OF SYMBOLS AND ABREVIATIONS	4
I	INTRODUCTION	5
II.	PURPOSE OF THE STUDY	8
III	DEFINITIONS AND TERMS	9
IV	CONSIDERATIONS TAKEN DURING THE PLANNING OF THE EXPERIMENT	10
V	SUBJECTS	13
VI.	METHODS	15
	1 General	15
	2. Bicycle ergometers	16
	3 Determining the capacity for physical work	16
	4 Calculating the work rate for training	19
	5 Statistical methods	20
VII.	EXPERIMENTAL PROCEDURE	21
VIII.	RESULTS	24
	1 The correlation between initial work capacity and measures of physical dimensions and activity	24
	2 The change in work capacity and its variation with the intensity frequency and duration of training	25
	1 Short time work capacity	25
	2 Prolonged work capacity	28
	3 The relationship between the capacity for prolonged and short time work and the change in this relationship	29
IX.	DISCUSSION	30
	1 Methodological problems	30
	1 The mode of expressing work capacity	30
	2 Conversion of maximum work time at a certain work rate into maxi mum work rate for a set work time	30
	3 Reliability in determining work capacity	32
	4 Intended and actual intensity	32
	5 Different ways of expressing intensity	33
	6 Room temperature	33
	7 Learning and habituation	34
	2. Relationships between initial work capacity and measures of physical dimensions and activity	35
	3 Change in work capacity	35
	1 The significance of initial work capacity	35
	2. The significance of intensity	36
	3 The significance of frequency	37
	4 The significance of the duration of the training session	

4 Training and heart rate 38
5 The relationship between increase in the capacity for short and prolonged work 40
6 Prediction of work capacity 41
7 The relationship between the amount of training and the effect of training 41
8 Comparison between the present study the study by Shephard, and the study by Davies and Knibbs 42
9 Regarding the suitability of expressing the increase of work capacity in percentage terms 45
10 The coefficient of regression in the relationship between log work time and log work rate 46
1 Initial slope as a function of measures of physical dimensions and activity 46
2. Change in slope as a function of training 46
11 The test subjects' subjective experiences 46
12. Choice of training program 47
X. ACKNOWLEDGEMENTS 50
XI. REFERENCES 51

ABSTRACT

NORDESJÖ L.-O *The effect of quantitated training on the capacity for short and prolonged work.* Acta physiol. Scand. 1974 90 Suppl. 405

A group of 27 subjects followed individual training programs on the bicycle ergometer for 8 weeks. The training programs were varied as to the intensity (50 75 or 100 % of the work rate that could be sustained the set time), frequency (1 3 or 5 times per week), and duration (15 60 or 120 minutes). After 4 weeks training, adjustments based on the increases in work capacity were made for the individual work rates.

Analysis of the results showed that the most important factor was the intensity of training, but frequency and duration could also be shown to be of significant importance. Some improvement of both short ($W_{max\ 6\ min}$) and prolonged ($W_{max\ 90\ min}$) work capacity was achieved even with the easiest program, but the greatest improvements followed training with maximum intensity frequency and duration. There was no significant relationship between initial work capacity and improvement, even if the influence of the intensity frequency or duration of training was eliminated.

LIST OF SYMBOLS AND ABBREVIATIONS

$\bar{x}$	a bar over a term denotes a mean
b	slope of regression line
b_n	slope of regression line at specified round of test
CH	chest-head lead
CR	chest-right arm lead
CPW	capacity for prolonged work (90 min)
CSW	capacity for short work (6 min)
D	duration of training session
ΔCPW	difference in capacity for prolonged work before and after training
ΔCSW	difference in capacity for short work before and after training
DLG	Dauerleistungsgrenze (see section I)
ECG	electrocardiogram
EPS	Erholungspulssumme (see section I)
F	frequency = number of training sessions per week
HR	heart rate (beats x min^{-1})
HR_{max}	heart rate at end of test for short time work capacity
HR_{work}	mean heart rate during training
I	intensity = highest work rate that can be sustained for a given time
LPI	*Leistungspulsindex (see section I)*
N	work rate (= load) during tests training
N_{PW}	work rate during tests for prolonged work capacity
N_{SW}	work rate during tests for short time work capacity
O_2-DLG	Dauerleistungsgrenze according to Hollmann (the value below which oxygen uptake d es not rise, irrespective of the duration of tim)
T	time
T_{PW}	work tim for test of the capacity for prolonged work
T_{SW}	work tim for test of the capacity for short work
V	gas volume per unit time
$\dot{V}O_2$	volume f oxygen per minute (= oxygen uptake per min)
$\dot{V}O_{2}max$	maximal oxygen uptake
W	work rate
W_{130}	work rate t heart rate f 130 beats per min
$W_{max\ x\ min}$	th highest work rate that can be sustained for a given time

Statistical terms

$\bar{x}$	mean
$\bar{d}$	mean difference or difference between two means
SD	standard deviation
n	number of subjects
s_e	standard deviation f residuals around the regression line
s_y^2	variance in the dependent variable
r	coefficient of correlation
$r_{xy.z...}$	coefficient of partial correlation
R	coefficient of multiple correlation
p	probability
*	probably significant ($p < 0.05$)
**	significant ($p < 0.01$)
***	highly significant ($p < 0.001$)

I INTRODUCTION

The human capacity for physical work varies within very wide limits. An individual's physical work capacity is chiefly determined genetically but can to a great extent be influenced by training as well as by inactivity (Cuthbertson 1929 Taylor *et al* 1949 Saltin *et al.* 1968).

There seems to be agreement that physical activity is beneficial for the individual, but as regards the prevention of coronary heart disease, for example, the benefit of physical activity has not yet been proved (Andrée Larsen and Malmborg 1971).

On the other hand, no definitive guidelines on the mode of training can be given. Various types of training, mainly interval, distance and circuit training all have their proponents. In a few studies different types of training have been compared (Clasing *et al* 1966 b Hollmann *et al.* 1966 Roskamm *et al.* 1966 b). The results were not quite conclusive, but it seemed as if distance training was equally efficient or slightly better than interval training.

Neither can any definitive guidelines be given on the amount of training necessary to give a sufficient increase in physical work capacity Several of the previous studies on training were of the cross-section type, studies in which athletes and non-athletes were juxtaposed for purpose of contrast. In longitudinal studies, where the subjects in training have been followed for 2—6 months, the maximal oxygen uptake has been reported to increase between 7 and 33 % (Robinson and Harmon 1941 Knehr *et al.* 1942 Taylor *et al.* 1949 Rowell 1962 Ekblom *et al.* 1968 Saltin *et al.* 1968 Saltin *et al.* 1969 Kilbom 1971 a, b and c).

There is a rather limited number of studies in which the authors have attempted to make an exact quantification of the training (Roskamm *et al.* 1966 a and b Siegel *et al.* 1970 Kilbom 1971 a and c). In two studies the intensity duration, and frequency of training have been varied, and the attempt has been made to describe the change in work capacity as a function of the training s intensity frequency and duration as well as of the initial work capacity (Shephard 1968 Davies and Knibbs 1971).

The above mentioned studies have all dealt with the effect of training on the capacity for short time work (2 to 10 min), either expressed as work rate at a set heart rate (W_{170}) (Roskamm *et al.* 1966 a and b), watt pulse (Roskamm *et al.* 1966 a and b), or as estimated (Shephard 1968) or measured oxygen uptake capacity (Siegel *et al.* 1970 Davies and Knibbs 1971 Kilbom 1971 b and c). A large number of tests have been devised in order to study the capacity for short time work (for references see Hahsman 1967 Blohmke 1969 Lange Andersen *et al.* 1971).

However from the standpoint of work physiology the capacity for prolonged work ought to be of equally as great an interest. This work capacity has been defined as the work rate an individual can maintain during eight hours without becoming exhausted, i.e., the processes of fatigue and recovery are in equilibrium (Karrasch 1951 Müller 1961). Since in work with large muscle groups it is probably factors which primarily set the limit, Müller and his associates

of the capacity for prolonged work could be obtained by recording the heart rate during a work day Heart rate was subsequently recorded both during occupational work and during exercise on a bicycle ergometer It was found that at a certain work rate, which was specific for each individual the heart rate, after an initial rise, remained constant (increase of less than 1 beat per min per hour Rohmert 1962) whatever the duration of work. The total recovery heart rate (Erholungspulssumme = EPS), i.e. the sum of the heart rate during the period of recovery minus the sum of the heart rate at rest during an equivalent period of time, also remained constant. Müller designated this work rate as Dauerleistungsgrenze (DLG) (Karrasch and Müller 1951).

Subsequent experiments showed that it was possible to determine DLG by means of three or four 10 (Müller 1955) or 60 (Rohmert 1962) minute work tests with different work rates and recording of EPS During work at the DLG-work rate the heart rate was seldom higher than 110 beats per minute, i.e., seldom more than 33—40 beats above the heart rate at rest (Müller 1961 Rohmert 1962). Since both methods are highly time-consuming, Müller (1950) introduced an index, designated as the Leistungspuls Index (LPI), as a more easily obtained measure. This is based upon the rate of increase in the heart rate when the work rate during an ergometer cycle test is raised every minute by 60 kpm x min^{-1} from 60 to 600 kpm x min^{-1}

In conducting spiroergometric studies, Hollmann (1963) found that during work at certain work rates the oxygen debt was higher after 20 minutes of work than it was after five minutes, while at other and lower work rates it remained the same regardless of whether the work lasted five or 20 minutes. The highest work rate which gave the same oxygen debt after 20 minutes as it did after five minutes was designated O_2 DLG This work rate is higher than the DLG defined by Müller and consequently results in a gra increase of heart rate. The O_2 DLG is approximately the same work rate as W_{130}, is the reason for Hollmann s proposal that endurance capability simply be mea red as W_{130}. In several studies involving military conscripts the mean value of W_{130} been shown to be between 600 and 702 kpm x min^{-1} (Clasing *et al.* 1966 a and b Roskamm *et al.* 1966 b)

Müller points out that one ought not to expect any relationship between a capacity for 8 hours of work and maximal oxygen uptake (1961). He bases his statement on observations made in conjunction with a study of a group of subjects which was very heterogeneous with respect to age. But Müller himself showed that there was a relationship ($r = 0.89$ $p < 0.001$) between the group mean values for LPI and maximal oxygen uptake for the subgroups who were 10 to 33 years old. In the original description of the LPI, based on 16 subjects without recorded age, Müller (1950) found a relationship between LPI and W[illegible] ($r = 0.71$ $p < 0.01$).

In a study of 15 young men Davies and Harris (1964) have also succeeded in showing a relationship between LPI and maximal oxygen uptake ($r = 0.56$, $p < 0.05$).

On the basis of calculations founded on daily caloric intake, caloric consumption during work, and maximal oxygen uptake, Bink (1962) has shown that there probably is a linear relationship between work time and absolute work rate when both are expressed as logarithms. Furthermore, a direct relationship between maximal oxygen up-

take and oxygen consumption in daily work has been shown for construction workers. In daily work the oxygen consumption was about 40% of the maximal (Åstrand, I. 1967).

Thus, there appears to be a relationship between the capacity for short time work and the capacity for work of longer duration.

In many instances, however work of long duration which results in a certain degree of exhaustion is of greater interest than work at DLG work rate. In these cases W_{130}, as suggested by Hollmann, is a conceivable work rate and a conceivable measure. At a heart rate of 130 beats per min the oxygen uptake is about 50 per cent of the maximal in young individual of about 25 years of age (Åstrand, I. 1960).

However well-trained individuals can sustain a balance between oxygen uptake and oxygen consumption at a higher relative work rate (work rate at which the oxygen uptake is a certain per cent of the maximal) than can untrained individuals (Åstrand and Rodahl 1970). For this reason W_{130} is not entirely suitable as a measure of the capacity for prolonged work. Furthermore, W_{130} like LPI has a relatively low reliability (Blohmke *et al.* 1966 Ehrenstein and Müller-Limmroth 1968 Blohmke 1969).

Rohmert (1962) has shown that there is a linear relationship between log maximal work time and log work rate (expressed as a multiple of DLG) for work within the time interval 8–197 minutes. Ahlborg (1966) studied work of approximately the same duration (2–240 min) and found equivalent correlations between log maximal work time and work rate (r = 0.90) and between log maximal work time and log work rate (r = 0.89). Consequently it is possible to study the capacity for prolonged work by methods which are designed on the basis of the relationship between maximal work time and work rate.

II PURPOSE OF THE STUDY

The present study proposes to determine

1 How the capacity for short time and prolonged work is influenced by training of varying intensity frequency and duration
2. If the relationship between the capacity for short time and prolonged work is changed as a result of training
3 To what degree of certainty the capacity for prolonged work can be predicted.

III DEFINITIONS AND TERMS

In this study every work task which leads to exhaustion will be designated as maximal, and the work rate which leads to exhaustion after t minutes will be designated $W_{max\ t\ min}$.

The capacity for short time work is defined as the highest work rate the test subject can sustain for 6 minutes ($W_{max\ 6\ min}$), and the capacity for prolonged work is defined as the highest work rate the test subject can sustain for 90 minutes ($W_{max\ 90\ min}$). The capacity for short time and prolonged work are hereafter abbreviated in the tables and equations as CSW and CPW respectively.

The intensity of a training task is given here as a percentage of the highest work rate the subject manages to sustain throughout the entire training session. If the test subject was completely exhausted by the end of the training session, the intensity has consequently been 100 %. In absolute terms this means that a given intensity for example 75 %, denotes different work rates if the period of training is to be, for example, 15 60, and *120* minutes respectively. (In comparing this study with others, where intensity is defined in other ways, for example as a percentage of $\dot{V}O_{2\ max}$, one must keep this definition in mind).

IV CONSIDERATIONS TAKEN DURING THE PLANNING OF THE EXPERIMENT

In as much as the primary interest of this experiment was tied to the effect of training on the capacity for prolonged work, it seemed natural to construct the training programs as so-called distance training i.e. continuous work at constant work rate. One reason for this choice is the fact that in a series of training experiments (Roskamm and Claasing 1967) distance training has yielded an equivalent or even better effect on W_{170} than has interval training.

Since training sessions of long duration could be expected to be more efficient than training sessions of short duration as regards the training of the capacity for prolonged work, it was decided that 2/3 of the training programs would involve training periods of very long duration, while for the sake of comparison 1/3 would be used for shorter periods. The duration of the training sessions was fixed at 120, 60 and 15 minutes respectively.

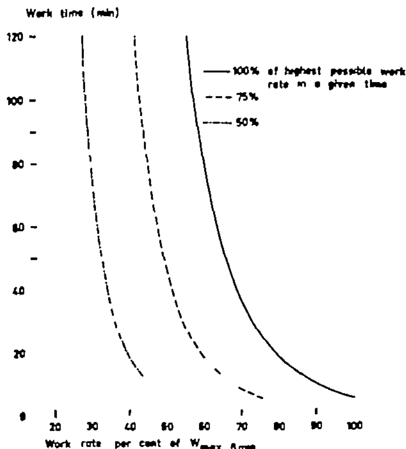
The results of previous studies (Michael *et al.* 1961, Åstrand, I. 1967) indicate that an individual manages to work for 8 hours without becoming appreciably tired at a work rate which is the equivalent of 35–40 % of the maximal oxygen uptake. Training at a lower work rate can therefore be expected to have a very low or no effect at all.

By means of the relationship between maximal work time and work rate and that between $W_{max\,6\,min}$ and $\dot{V}_{O_2\,max}$ (Nordesjö 1974 a) as well as Åstrand's nomogram (1960) one can convert the work intensity expressed as work rate in per cent of maximal work rate for a given duration of work, into intensity expressed as oxygen uptake per cent of the maximal oxygen uptake.

An example: assume that $W_{max\,6\,min}$ for an individual is 1500 kpm x min^{-1}. His maximal oxygen uptake is then calculated to be about 3.2 l x min^{-1} ($\dot{V}_{O_2\,max}$ = 0.001685 $W_{max\,6\,min}$ + 0.7208). If he works at 80% of $W_{max\,6\,min}$ i.e. 1200 kpm x min^{-1} the oxygen uptake should be about 2.8 l x min^{-1} i.e. an oxygen uptake which is about 87.5 % of the subject's maximal oxygen uptake.

According to the relationship between log work time and log work rate as stated by Rohmert (1962) and Tornvall (1963), the highest work rate an individual can sustain for 120 min ($W_{max\,120\,min}$) corresponds to 55 % of the highest work rate the individual can sustain for 6 minutes ($W_{max\,6\,min}$). This means that 50 % of $W_{max\,120\,min}$ = 27.5 % of $W_{max\,6\,min}$ = 37 % $\dot{V}_{O_2\,max}$, which is equivlent to the above mentioned level reported by Michael *et al.* (1961) and Åstrand, I. (1967).

Since it was considered of less interest to train the test subjects at work rates which could hardly be expected to yield any increase in work capacity, the lowest intensity was fixed at 50 % of the highest work rates which the test subject could be expected to sustain for 15, 60 and 120 minutes respectiely. At the same time, certain test subjects were to train at the highest possible intensity (100%), while other trained at 75%. Fig. 1 illustrated the proportional relationship of the chosen intensities to $W_{max\,6\,min}$ and $\dot{V}_{O_2\,max}$ for training periods of different duration.



Work rate per cent $\dot{V}_{O_2\,max}$ of $W_{max\ 8\ min}$

		40	49		57	65	73	82	90	1000 kpm min^{-1}
29		38	48		57	66	77	88	99	1500 kpm min^{-1}
28	35	38	47	50	57	69	81	91	100	2000 kpm min^{-1}

Fig. 1 Relationship between work rate as percentage of physical work capacity expressed as $W_{max\ 8\ min}$ and work time according to the equation $T = 6 \times 10^{-14}$ (work rate in % of $W_{max\ 8\ min}$)^b when $b = -5$. The lines represent 50, 75, and 100% of the highest work rate that can be sustained for any given time. At the bottom of the figure is given the oxygen uptake, expressed as a percentage of maximal oxygen uptake, equivalent to the work rate when $W_{max\ 8\ min}$ is 1000, 1500, and 2000 kpm x min respectively

The frequency of training was also arranged in three steps 1 3 or 5 times a week. In this way 27 different training programs (see Fig. 2) were obtained, and every ject was to train for 8 weeks according to one of these programs.

Duration of training session (min)		15			60			120		
Frequency of training (times per week)		1	3	5	1	3	5	1	3	5
Intensity of training (%)	50	0	0	X	0	0	X	0	X	X
	75	0	0	0	0	0	X	X	●	X
	100	X	X	X	X	X	X	X	X	X

X = Spring 1969
0 Spring 1970
● = Autumn 1970

Fig. 2. Training programs.

V SUBJECTS

A total of 29 men applied after the announcement of the exeriment. The reason for par ticipation in the experiment was the possibility for improved physical fitness as a result of the training. The test subjects were also offered a modest renumeration which covered travelling expenses and also served as an incentive for further participation, in as much as this compensation would only be given out after completion of the training program.

Of the 29 test subjects, 2 were actively engaged in ahtletic competition and were therefore excluded from the series. One test subject became ill while the tests were in progress, and a replacement underwent the equivalent training program 6 months later

Data on the 27 test subjects who took part in the whole program are given in Table I This table shows that the test subjects, whose average age was 22.5 years, were slightly taller (unpublished data) and heavier (*Ahlborg et al. 1972*) than 18 year-old military registrants (Linroth 1969) but that their capacity for physical work was significantly ($0.05 > p > 0.01$) higher This was probably because the groups consisted of volunteers.

Twenty-four test subjects were full-time university students, and three others held ac tive but very light jobs. The majority of the university students worked during the summer—6 to 9 months before the beginning of the experiment—in various occupations, ranging from heavier manual labour to desk jobs. Five test subjects were physically inactive even during their spare time seventeen played tennis, rode, or did some cross-country *running* on occasion, i.e., *were somewhat* active, *and five subjects were more*

TABLE I. Physical characteristics f the subjects (27) and some data on physical activity and capacity compared with data for a representative sample of Swedish boys of 18 (n 1719–1789).

	The subjects $\bar{x}$	SD	Range	Boys of 18 $\bar{x}$
Age, years	22.7	1.8	20 0–25.8	18.5
Height, cm	180.7	6.5	170.5–194 0	177.2
Weight, kg	67.9	8.0	55.2–84.5	66 4
Heart volume, ml	740	113	525–1070	673[a]
Heart rate at rest, beats x min^{-1}	67 7	7.3	54–84	74.9
Physical activity				
occupational	1.1	0.3	1–2	–
recreational	2.0	0.6	1–3	–
W_{max} 6 min kpm x min	1556	235	1107–2114	1451
Heart rate at end f work test, beats x min^{-1}	190.9	8.2	174–204	193.6

[a]Calculated from chest photo fluorogram taken in the standing position

regularly engaged in physical exercise, in the form of swimming, cross-country running or bicycling. None of them competed actively in athletics, but one had been an active cross-country runner the year before. Nineteen test subjects were non-smokers, although two of them used snuff. Three smoked 1—10 cigarettes a day and five smoked 11—20 cigarettes a day.

Due to limited capacity, 16 test subjects were examined in the spring of 1969, 10 in the spring of 1970, and 1 (the replacement for the test subject who became ill in the spring of 1970) in the autumn of 1970.

According to their own reports, the test subjects' physical activity and fitness were basically the same during the 6 months prior to the training study. Fifteen of the 16 test subjects who trained in the spring of 1969 had in the autumn of 1968 participated in experiments regarding method reliability (Nordesjö 1974 b). Their own reports can therefore be supplemented by the test results from September and December 1968, and from the period immediately prior to the training. The mean value for $W_{max\,6\,min}$ was 1634, 1647, and 1630 kpm x min^{-1} respectively. Heart volume and weight were entirely unchanged: 728 ml and 67.5 kg, 736 ml and 67.2 kg respectively.

Apart from the fact that one test subject began to train by running somewhat during the last 14 days, the test subjects' physical activity was unchanged both at work and during free time.

VI METHODS

1 General

Information regarding the test subjects smoking habits and physical activity during spare time and while at work, or the equivalent (studies), was obtained by means of a questionnaire based upon one which has been used previously in studies of training (Saltin and Grimby 1968 Kilbom *et al.* 1969). Smoking was registered in terms of the number of cigarettes per day and physical activity was graded according to the following.

Physical activity during spare time

1 Almost completely inactive reading, TV movies, etc.
2 Some physical activity during 4–6 hours a week riding a bicycle or walking to work, walking with the family fishing, working in the garden, playing table tennis, bowling
3 Regular physical activity such as running, callisthenics, tennis, swimming
4 Regular hard physical training several times a week for competition in running events, soccer handball, etc.

Physical activity while at work or the equivalent

1 Predominantly sedentary work studies, desk work, watchmaking, sitting assembly worker (light goods)
2 Sitting or standing work, some walking shopclerk, office worker light tool and machinery worker foreman, teacher or instructor
3 Walking and some handling of material mailman, waiter construction worker heavy tool and machinery worker
4 Heavy manual work lumberjack dockworker stone mason, farmworker unskilled labourer fisherman.

Height was taken to the nearest 0.5 cm, with the test subject standing without shoes.

Weight was taken to the nearest 0 1 kg, with the test subject clad only in gymn shorts.

ECG was registered at rest and after 8 minutes in standing position with 11 leads on an Elema mingograph 42 or 34 The speed of the paper was checked by measuring the length of the paper during a set period of time. At least 20 complexes were registered by ECG during work. At rest and while standing leads CR_{1-7} were registered and during the initial work test leads CH_{2-7}. During the subsequent work test and training sessions the heart rate was registered by means af lead CR_4.

Heart volume was calculated according to the formula proposed by Kjellberg *et al.* (1951) from anterio-posterior and lateral roentgenograms taken with the tube at right angles to the table. The subject was placed in a supine position with cranially extended arms. No consideration was paid to the phase of the heart cycle. Owing to technical reasons roentgenograms were not taken on all subjects before and after training. On

those that were, there was no significant difference ($d = + 17\ 46$ ml, $p > 0.2$, $n = 16$) between the two results, and no relationship could be discerned between the difference in heart volume on the one hand and the frequency intensity or duration of the training on the other Since the difference was so small the mean of the individual values before and after training are taken to represent each subject s heart volume, except for the cases where there only exists one value.

2. Bicycle ergometers

Tests and training were conducted on 2 slightly modified electrically braked bicycle ergometers, Elema-Schönander model AM 368 (Holmgren and Mattson 1954). The calibrations of the bicycle ergometers were checked at the factory before, during and after each of the test series (spring 1969 spring 1970 and autumn 1970). No deviations from the calibration report were noted in so doing. During the standard calibration procedure the ergometer is run in the test bench for about 10 min at a low load. A nominal load (= work rate) at 50 kpm x min $^{-1}$ is then set and after 1 min the torque at 60 r p.m. is recorded on a balance and the actual load is calculated. The nominal load is then increased to 100 kpm x min $^{-1}$ the torque recorded after 1 min and so on until 2000 or 2050 kpm x min^{-1} The procedure takes 17 min and is then repeated for 45 and 75 r p.m.

As can be seen from Table 2, the actual load falls below the nominal load during prolonged work on higher loads. Before modification the actual load was about 30 kpm x min below the nominal 1800 kpm x min^{-1} after 10 min work, and 70 kpm x min^{-1} below nominal after 60 min work.

In as much as the highest measureable work rate on ergometer model AM 368 is 2050 kpm x min a test subject with a very high initial work capacity had to perform tests and training on ergometer cycle model 361 On this model the actual work rate increases during work performed at high levels (see Table 2). The values of the above mentioned test subject have been corrected on the basis of the results of trials in the test bench on the work rates which were used during tests and training.

On both ergometer the pedal length was fixed at 17.5 cm. The sadle height was adjusted in such a way that the test subjects legs would be flexed about 5 degreas at the lowest point of revolution.

3 Determining the capacity for physical work

All work tests were conducted without warming-up and continued until the test subject could no longer maintain the prescribed number of revolutions, 60 r p.m. At the end of the short time work tests the test subject was completely exhausted and had a high heart rate. However during the prolonged work tests it was more difficult for the examiner to determine whether the test subject was completely exhausted or not. In some

TABLE 2. Deviations from indicated loads in kpm x min⁻¹ at 60 r.p.m. for the ergometers used in the present study

Ergometer model	361				368 Modified				
No	103				563				666
Nominal load	500	1000	1500	2000	500	1000	1500	2000	1500
Time min									
0	0	0	0	−20	5	10	0	0	0
5	3	11	13	−3	−2	−5	−3	−30	−20
10	6	17	23	8	−5	−15	−7	−40	−30
15	7	25	26	18	−6	−20	−25	−35	−30
20	7	25	33	40	−8	−20	−25	−30	−30
30	8	28	53	65	−8	−25	−25	−30	−30
40	8	28	68	100	−8	−25	−25	−20	−30
50	8	32	78	135	−10	−25	−25	−20	25
60	8	32	83	170	−10	−25	−30	−15	−25
70	8	40	93	200	−10	−25	−30	−10	−25
80	10	40	103	220	−10	−25	−30	0	− 5
90	10	40	108	235	−10	−25	−30	+10	−22
100	10	46	108	255	−12	−25	−30	+10	−22
110	10	46	–	280	−12	−25	−30	+15	−22
120	10	49	–	230	−12	−20	−25	+20	−22

cases the test subject appeared to be completely exhausted, in others the heart rate was nearly or equally as high as it was during any of the subjects short time work tests. Again, in other cases where the test subject still had a moderate heart rate the test subject broke off the test with the explanation that his legs could take no more. In doubtful cases a new test was carried out a few days later Both during the prolonged work tests and during training the test subject was allowed to drink *ad lib.* The amount of water consumed (50—1500 ml) varied with the intensity and duration of the work and room temperature. Loss of water due to sweating and ventilation were, however not fully compensated. For example, during 60 min of training with 100 % intensity at a room temperature of 21°C the subject drank 440 ml of water but lost 0 4 kg in weight. The following work tests were used

1 During the preliminary study a work test was used in which the work rate was initially 300 or 600 kpm x min⁻¹ and was raised by 300 kpm x min⁻¹ every 6 minutes until exhaustion occurred. A value of $W_{max\,6\,min}$ was calculated by weighing together the times for the different work rates in terms of a formula stated by Tornvall (number 1 below) according to principles presented previously (Nordesjö 1974 b).

2. In determining the capacity for short time work (CSW) the work test of Tornvall (1963) was used, i.e., a work test with constant high work rate where the work time is allowed to vary between 1 and 18 min. The result ($W_{max\,6\,min}$) is calculated according to the following formula

$$\log W_{max\,6\,min} = \frac{\log T_{SW} - \log 6}{-b} + \log N_{SW} \qquad (1)$$

those that were, there was no significant difference (d = + 17 46 ml, $p > 0.2$, $n = 16$) between the two results, and no relationship could be discerned between the difference in heart volume on the one hand and the frequency intensity or duration of the training on the other Since the difference was so small the mean of the individual values before and after training are taken to represent each subject s heart volume, except for the cases where there only exists one value.

2. Bicycle ergometers

Tests and training were conducted on 2 slightly modified electrically braked bicycle ergometers, Elema Schönander model AM 368 (Holmgren and Mattson 1954). The calibrations of the bicycle ergometers were checked at the factory before, during and after each of the test series (spring 1969 spring 1970, and autumn 1970). No deviations from the calibration report were noted in so doing. During the standard calibration procedure the ergometer is run in the test bench for about 10 min at a low load A nominal load (= work rate) at 50 kpm x min^{-1} is then set and after 1 min the torque at 60 r p.m. is recorded on a balance and the actual load is calculated. The nominal load is then increased to 100 kpm x min^{-1} the torque recorded after 1 min and so on until 2000 or 2050 kpm x min^{-1} The procedure takes 17 min and is then repeated for 45 and 75 r p m.

As can be seen from Table 2, the actual load falls below the nominal load during prolonged work on higher loads. Before modification the actual load was about 30 kpm x min^{-1} below the nominal 1800 kpm x min^{-1} after 10 min work, and 70 kpm x min^{-1} below nominal after 60 min work.

In as much as the highest measureable work rate on ergometer model AM 368 is 2050 kpm x min a test subject with a very high initial work capacity had to perform tests and training on ergometer cycle model 361 On this model the actual work rate increases during work performed at high levels (see Table 2). The values of the above mentioned test subject have been corrected on the basis of the results of trials in the test bench on the work rates which were used during tests and training.

On both ergometer the pedal length was fixed at 17.5 cm. The sadle height was adjusted in such a way that the test subjects legs would be flexed about 5 degrees at the lowest point of revolution.

3 Determining the capacity for physical work

All work tests were conducted without warming-up and continued until the test subject could no longer maintain the prescribed number of revolutions, 60 r p.m. At the end of the short time work tests the test subject was completely exhausted and had a high heart rate. However during the prolonged work tests it was more difficult for the examiner to determine whether the test subject was completely exhausted or not. In some

$$b = \frac{\log T_{PW} - \log T_{SW}}{\log N_{PW} - \log N_{SW}} \qquad (4)$$

T_{PW} = work time in min during the prolonged work test
T_{SW} = work time in min during the short time work test
N_{PW} = work rate in kpm x min^{-1} during the prolonged work test
N_{SW} = work rate in kpm x min^{-1} during the short time work test.

(b) with a weighted mean value for the coefficient of regression for the 27 test subjects on each test occasion. This value is calculated according to the following formula

$$\bar{b} = \frac{\sum_{1}^{m} \sum_{1}^{n} (\log N - \overline{\log N})\ (\log T - \overline{\log T})}{\sum_{1}^{m} \sum_{1}^{n} (\log N - \overline{\log N})^2} \qquad (5)$$

where m = the number of individuals, n = the number of observations per individual, T = work time, and N = work rate (see Snedecor and Cochran 1967 pp. 435 and 443).[1]

Since the difference between these two methods of calculating is very small (see the discussion), only the results of method b) are presented in the following.

4 Calculating the work rate for training

In calculating the work rate at which the test subject should be training the results of both short time and prolonged work tests were utilized. By inter or extrapolation it was possible to calculate the highest work rate the test subject could manage to sustain throughout the entire training session, i.e., in 15 60, or 120 minutes respectively The test subject was to train at 50, 75 or 100 % of this work rate depending upon the training program he had been assigned to (In practice, inter or extrapolation was effected in such a manner that the coefficient of regression calculated for the individual was introduced in equation (4) together with the logarithms for work time and work rate in the test of short time work capacity and T_{PW} was replaced by the length of the training period—15 60, or 120 min. Consequently the unknown factor which can now be calculated will designate the highest work rate the test subject can sustain for 15 60, or 120 minutes.)

[1] In the case where the individual' regression is based upon two points (2), equation 5 can be simplified to

$$b = \frac{\Sigma \log T_{PW} - \Sigma \log T_{SW}}{\Sigma \log N_{PW} - \Sigma \log N_{SW}}$$

Current statistical methods were used (Snedecor and Cochran 1967).

To describe a variable the mean value ($\bar{x}$, $\bar{d}$ or $\bar{y}$), the standard deviation (SD), and sometimes the minimum and maximum value (here called range) were used.

Simple linear correlation coefficients (r) have been used to describe the association between two variables.

Linear regression analysis was used to describe one variable (y) as a function of one or more other variables (x or x_1 x_2 x_4) i.e. $y = a + bx$ or $y = a + b_1 x_1 + b_2 x_2 + \ldots + b x$ where a and b are regression coefficients. In the text b is sometimes called slope.

In connection with these functions (sometimes mentioned as equations) the residual standard deviation (s_e) and multiple correlation coefficient (R) is given. The residual standard deviation is the square root of that part of the variance of y (s_y^2) that is not accounted for by the function. The square of the multiple correlation coefficient equals the fraction of the variance of y that is accounted for by the function.

Sometimes partial correlation coefficients ($r_{12.3}$, $r_{12.34}$ etc.) are also given. The partial correlation $r_{12.34}$ is the correlation between variables 1 and 2 after elimination of the influence of variables number 3 and 4

The hypothesis tested is always a null hypothesis, meaning that there is no difference (alternatively correlation etc.) in the (hypothetical) population.

The following table gives the different levels of significance that were used, how they are denominated in the text and how significant correlations, differences, variables etc. are marked in Tables 3–6, 11 and 14

Probability (p) of rejecting the hypothesis when it is true	Denomination in the text	Mark in tables
< 0.001	Highly significant	* *
< 0.01	Significant	**
< 0.05	Probably significant	*
$\geqslant 0.05$	Not significant	(no mark)

In treating the data, a stepwise regression program, BMD 02R, University of California 1971 based upon Efroymson (1960), has been used in order to obtain correlations and regressions.

VII EXPERIMENTAL PROCEDURE

During a preliminary visit the test subject was to fill in a questionnaire regarding health and physical activity and undergo a general examination which covered a record of individual height, weight, sedimentation rate, Hb, heart volume, blood pressure at rest, ECG at rest, an orthostatic test as well as a simple physical examination, the purpose of which was to eliminate cardiopulmonary disease and the presence of upper respiratory tract infections. After this a preliminary work test was conducted. (Regarding the outline of the test, see section VI. 3 1).

Once the examination showed that nothing hindered the test subject from participating in the study he drew lots (without replacement) and received his assignment to one of the 27 training programs (see Fig. 2).

A few days after the preliminary examination the initial short time work capacity (CSW) was determined (see Fig. 3). Here the test subject was to work at a work rate

Day 1	– Preliminary tests and graded work test	
2	– Determination of heart volume	
etc	– Test on the capacity for short time work	(CSW_1)
	–	
	– Test on the capacity for prolonged work	(CPW_1)
	–	
	4 week training	
	–	
	Test on the capacity for short time work	(CSW_2)
	–	
	– Test on the capacity for prolonged work	(CPW_2)
	–	
	–	
	4 weeks training	
	– Determination of heart volume	
	Test on the capacity for short time work	(CSW_3)
	Test on the capacity for prolonged work	(CPW_3)

Fig. 3 Experimental procedure

which was consistent with the result of the preliminary work test. However the work rate was rounded off so as to be divisible by 50

Two days after the test of short time work capacity the initial prolonged work capacity (CPW_1) was determined. (Regarding the outline of the test and choice of work rate, see section VI.3.3).

After another two days time training began at the frequency duration, and intensity which had been determined for the test subject by the lottery process.

The training continued for four weeks without changes in the work rates. To the extent that work capacity increased during these four weeks the intensity of the training was relatively decreased, in as much as the absolute work rate for training remained constant. In order to determine possible changes in the work capacity and to be able to adjust the work rates for training in such a way that the intensity was once again intended, a second test of short time (CSW_2) and prolonged work capacity (CPW_2) was conducted 2 and 4 days respectively after the last training session in the fourth week of training.

Since it was expected that the test subjects work capacity had increased, a work rate which was somewhat higher than the work capacity measured before the beginning of training (CSW_1) was used during the second test of short time work capacity (CSW_2). (For the test subject who had the hardest training program—100 %, 120 min and 5 times a week—the work rate was about 200 kpm x min^{-1} higher for the test subject with the easiest program—50%, 15 min and 1 time per week—the work rate was about 50 kpm x min^{-1} higher)

The appropriate work rate for the second test of prolonged work capacity was calculated according to equation (2), see section VI.3.2, on the basis of work time and work rate from the second test of short time work capacity and on the individual coefficient of regression, which was calculated from the tests which preceeded training (b_1).

After this the test subject trained for four more weeks at the work rates which were calculated on the basis of the results from the second tests of short time and prolonged work capacity Two and four days respectively after the end of training, short time (CSW_3) and prolonged work capacity (CPW_3) were once again determined. The work rates for these tests were determined in the same way as above for CSW_3 50–200 kpm x min^{-1} were added to the midway value of short time work capacity (CSW_2), and for CPW_3 the work rate was based upon work rate and work time for the third test of short time work capacity and the slope obtained at the midway test (b_2). However during the last test of prolonged work capacity (CPW_3) the test subject was set to work at a work rate which was about 50 kpm x min^{-1} higher than had been calculated. The reason for this was a desire to counteract any conscious or unconscious tendency on the part of the test subject to carry out an exactly 90 min long work test. The average work time was also 13 min shorter than during the first test occasion, and this corresponds rather exactly to the difference between estimated and applied work rate.

The method of procedure used here—one which meant that the work rate during short time work tests was chosen with respect to the result of the preliminary test and increases in work rate according to a set pattern—yielded the desired results. The

average work time in minutes was 6.0 (SD 1.5 range 3 4–10.9), 8.8 (SD 3.6, range 4.9–16.5), and 7 1 (SD 3.0, range 4 1–15 4) in tests before, during, and after training. The two, very long work times during the second and third test occasions resulted from the fact that one test subject had to work at a lower work rate than was desirable, since the highest work rate which could be registered on ergometer AM 368 was 2050 kpm x min^{-1}

The procedure also led to the desired results during the prolonged work tests. The average work times in minutes were 92.5 (SD 28.3 range 47.2–151 6), 95.3 (SD 38.7 range 50.3–206.2), and 79.3 (SD 19.0 range 50.0–124.0) in tests before, during, and after training.

The intention was to carry out tests of short time and prolonged work capacity during the morning hours and, where possible, at the same time for every individual. This was not entirely successful, in as much as the test subjects had other important activities.

The whole series of tests took a total of 10–12 weeks to complete. As a result of the very long period of examination and training, which did not come to an end until during the summer it was not entirely possible to control the room temperature. On most of the test occasions the temperature was between 20 and 24°C (22.5 ± SD 1 0), but during the last prolonged work test in the spring of 1969 the temperature was often 24–25°C (23.9 ± SD 1.2). During the first half of the training period the temperature was approximately 21°C, but during the last two weeks of training the average temperature was 24 9°C (spring, 1969) and 23.3°C (spring, 1970). An electric fan was used in the room when the temperature rose above 22°C.

During the heat waves the attempt was made to carry out the training and the tests during the morning hours, before the heat lowered the test subjects motivation.

The relative humidity increased during spring 1969 and 1970 from 43 % (SD 3 1 %) during the first test period to 50 % (SD 3.5 %) during the last test period. During the first period of training the relative humidity was 42 % (SD 1 1 %) and during the second 47 % (SD 1.8 %). In the autumn of 1970 the relative humidity fell slightly from 45 to 41 %.

VIII RESULTS

1 The correlation between initial work capacity and measures of physical dimensions and activity

The initial work capacity varies with heart volume and to a certain extent with weight and height, as shown in Table 3 (It is a pure coincidence that initial work capacity also varies with the frequency of training, since the training programs were assigned to the test subjects by a drawing of lots.)

The initial work capacity is better explained by heart volume than by weight and height combined and is equally well explained by heart volume alone than by heart volume and weight or height combined.

If the initial work capacity is expressed per kg of body weight, one quite naturally obtains a lower relationship with weight, height and heart volume (Table 3). On the

TABLE 3 The covariation between initial work capacity and anthropometric measures and other data (simple and multiple correlation coefficients)

Variable	*Absolute work capacity*		*Work capacity per kg body weight*	
	CSW	CPW	CSW	CPW
Age	0.18	0.16	−0.12	−0.09
Height	0.36	0.28	−0.20	−0.20
Weight	0.50**	0.45*	−0.28	−0.21
Heart volume	0.69***	0.76***	0.15	0.31
Physical activity:				
occupational	0.09	0.21	0.34	0.46*
recreational	0.32	0.20	0.64***	0.46*
oking	−0.23	−0.33	−0 25	−0.35
frequency	0.50 *	0.44	0.38	0.33
duration of session	−0 07	−0.18	0.25	0.30
intensity	0.30	0.20	0.09	0.02
Height²	0.36	0.28	−0.20	−0.20
Body surface area	0.50**	0.44*	−0.26	−0.26
Height and weight	0.50*	0.45	0.28	0.22
Hearth volume and weight	0.69**	0.77**	0.58**	0.70***
Heart volume and occupational physical activity	0.69**	0.77***	0.36	0.53*
Heart volume and recreational physical activity	0.79***	0.81***	0.67***	0.58**
Smoking and occupational and recreational physical activity	0 34	0.38	0.73***	0.66**
Heart volume, smoking, occupational and recreational physical activity	0.80**	0.81 **	0.71*	0.67 *

CSW = capacity for short work
CPW = capacity for prolonged work

TABLE 4 Work rate, heart rate at the end of work test, work capacity and the coefficient of regression of log work time on log work rate during the three test periods for the capacity for short-time work (CSW) and for prolonged work (CPW) Test period I = 4 and 2 days before training, test period II = during training, test period III = 2 and 4 days after training. Significance of the mean of individuals differences between various test periods are given in the last three columns.

Test period	I	I	II	III	I–II	I–III	II–III
Capacity for short work (CSW)							
Work rate	$\bar{x}$ SD	1565 237	1638 238	1779 252	***	***	***
kpm x min^{-1}	range	1050–2050	1280–2137	1350–2338			
Heart rate	$\bar{x}$ SD	191 9	191 9	190 9			
beats x min^{-1}	range	168–204	172–206	170–204			
W_{max} 6 min	$\bar{x}$ SD	1556 235	1714 275	1813 300	***	***	***
kpm x min^{-1}	range	1107–2114	1294–2243	1375–2331			
Capacity for prolonged work (CPW)							
Work rate	$\bar{x}$ SD	991 172	1157 236	1287 247	***	***	***
kpm x min^{-1}	range	650–1420	750–1650	900–1756			
Heart rate	$\bar{x}$ SD	179 13	175 13	181 11			**
beats x min^{-1}	range	149–200	153–194	158–202			
W_{max} 90 min	$\bar{x}$ SD	988 173	1156 213	1258 227	***	***	***
kpm x min^{-1}	range	662–1365	781–1587	861–1662			
Coefficient	$\bar{x}$ SD	−5.91 1.23	−7.07 1.34	−7.36 1.08		***	
of regression	range	−4.13/−8.88	−4.24/−10.44	−5.69/−9.52			

other hand, the test subject s occupational and recreational physical activity correlate better with initial work capacity expressed per kg of body weight than when taken in absolute terms.

2. The change in work capacity and its variation with the intensity frequency and duration of training

The work capacity after training differs significantly from the values obtained prior to training. There is no significant difference in the heart rate at the end of work (see Table 4)

2.1 Short time work capacity

The absolute change in (absolute) short time work capacity is primarily due to the intensity of training and consequently the total amount of work during training (see Table 5). The frequency of training has also a certain importance, while the covariation with the duration of training is not significant. (The correlations are the same if the change in work capacity is expressed per kg of body weight or as a percentage of the initial value, and therefore these variants are excluded in the following.) Following multiple regres

TABLE 5 Covariation between change in work capacity and the frequency duration and intensity of training, initial work capacity and some other variables (simple and multiple coefficients of correlation)

Variable	Change in CSW absolute	per kg b wt.	%	Change in CPW absolute	per kg b. wt.	%
Training: frequency (actual)	0.36	0.31	0.23	0.42	0.37	0.24
duration of session	0.18	0.23	0.16	0.23	0.32	0.29
intensity (actual)	0.60***	0.53**	0.55**	0.63***	0.55**	0.54**
Intensity as mean work rate $_{train}/W_{max}$ 6 min	0.33	0.28	0.30	0.28	0.20	0.15
Intensity as HR_{work}/HR_{max}	0.32	0.25	0.31	0.28	0.18	0.19
Mean work rate during training	0.44*	0.34	0.30	0.43*	0.31	0.19
Total work during training	0.60***	0.58**	0.48*	0.58**	0.57**	0.41*
Initial CSW	0.31	0.19	0.04	0.39*	0.26	0.07
Initial CPW	0.22	0.13	0.02	0.11	−0.02	−0.24
Initial CSW per kg b.wt.	0.19	0.24	0.03	0.31	0.39*	0.11
Initial CPW per kg b wt.	0.13	0.20	0.02	0.03	0.10	−0.23
Frequency and intensity of training	0.71**	0.63**	0.61**	0.78***	0.69***	0.60**
Frequency intensity and duration of training	0.74***	0.67**	0.63**	0.82***	0.76***	0.67**
Frequency intensity and duration of training and initial work capacity	0.74**	0.69*	0.70**	0.84***	0.81***	0.83***
Age	0.13	0.04	0.10	0.09	−0.03	0.05
H-i	−0.04	−0.20	−0.14	0.05	−0.15	−0.03
	0.17	−0.06	0.01	0.15	−0.12	−0.03
volume	0.28	0.13	0.10	0.17	−0.01	−0.10
Physical activity occupational	0.32	0.42*	0.31	0.14	0.22	0.05
Physical activity recreational	0.07	0.14	0.00	0.14	0.23	0.05
Smoking	0.17	0.15	0.25	0.19	0.15	0.32

CSW = capacity for short work
CPW = capacity for prolonged work

sion analysis the two most important factors of the training program—intensity and frequency—account for 51 % of the variance of the change of short time work capacity It does not prove worthwhile to include duration or initial work capacity in the equation (see Table 6).

In Table 7 the increase in short time work capacity for the different training programs has been calculated according to equation 3 in Table 6, and in Table 8 the increase in short time work capacity as a function of various combinations of training programs and initial short time work capacity has been calculated according to equation 4 in Table 6.

TABLE 6 Multiple regression between change in work capacity and the intensity frequency and duration of training and initial work capacity

Eq no.	Regression equation	r or R	s_e
	Change in capacity for short work (CSW) =		
1	$-61 + 3.99\,I^{**}$	0.60^{**}	104
2	$-171 + 4.14\,I^{***} + 32.7\,F^{*}$	0.71^{***}	93
3	$-207 + 4.14\,I^{***} + 32.4\,F^{**} + 0.5\,D$	0.74^{***}	91
4	$-156 + 4.38\,I^{***} + 36.5\,F^{*} + 0.5\,D - 0.051\,CSW_1$	0.74^{**}	93
	Change in capacity for prolonged work (CPW) =		
5	$-74 + 4.34\,I^{***}$	0.63^{***}	103
6	$-206 + 4.51\,I^{***} + 39.2\,F^{**}$	0.78^{***}	85
7	$-254 + 4.55\,I^{***} + 38.8\,F^{***} + 0.7\,D$	0.82^{***}	80
8	$-121 + 4.92\,I^{***} + 48.3\,F^{**} + 0.6\,D - 0.184\,CPW_1$	0.84^{***}	76

I = intensity as the work rate in per cent of the highest work rate the subject can sustain during the training session
F = no of training sessions per week
D = duration of training session in min
CSW_1 = capacity f r short time work (W_{max} 6 min) before training in kpm x min [1]
CPW_1 = capacity for prolonged work (W_{max} 90 min) before training in kpm x min [1]
r = linear coefficient of correlation
R = multiple linear coefficient of correlation
s_e = standard deviation of residuals round the regression line

TABLE 7 Predicted absolute change in work capacity as a function of the training program in kpm x min [1] (within brackets change in per cent) Duratio is expressed in min, frequency in times per week and intensity as the work rate in per cent of the highest work rat that can be sustained during the training session.

Duration		15			60			120	
Frequency	1	3	5	1	3	5	1	3	5
Intensity									
	Change in capacity for short work (CSW)								
50	42 (3)	106 (7)	171 (11)	66 (4)	131 (8)	196 (13)	98 (6)	163 (11)	228 (15)
75	146 (9)	211 (14)	276 (18)	170 (11)	235 (15)	300 (19)	203 (13)	268 (17)	332 (21)
100	250 (16)	315 (20)	380 (24)	274 (18)	339 (22)	404 (26)	307 (20)	372 (24)	437 (28)
	Change in capacity for prolonged work (CPW)								
50	23 (2)	100 (10)	178 (18)	55 (6)	133 (13)	210 (21)	98 (10)	175 (18)	253 (26)
75	137 (14)	214 (22)	292 (30)	169 (17)	246 (25)	324 (33)	212 (21)	284 (29)	367 (37)
100	250 (25)	328 (33)	406 (41)	283 (29)	360 (36)	438 (44)	325 (33)	403 (41)	481 (49)

TABLE 8. Predicted absolute change in the capacity for short time work (CSW) in kpm x min [1] as a function of the intensity (in %) and frequency (times per week) of training and initial CSW (kpm x min [1]) when training 60 min per session (eq no 4 in Tabl 6) In order to calculate equivalent values when training 15 or 120 min per session subtract 23 or add 31 kpm x min [1] respectively to the given values.

Intensity[a]		50			75			100	
Frequency	1	3	5	1	3	5	1	3	5
Initial CSW									
1000	80	153	226	189	262	335	299	372	445
1100	75	148	221	184	257	330	294	367	440
1200	70	143	216	179	252	325	289	362	435
1300	65	138	211	174	247	320	284	357	430
1400	60	133	206	169	242	315	279	352	424
1500	55	128	201	164	237	310	274	347	419
1600	50	123	196	159	232	305	269	341	414
1700	45	118	190	154	227	300	263	336	409
1800	40	112	185	149	222	295	258	331	404
1900	34	107	180	144	217	290	253	326	399
2000	29	102	175	139	212	285	248	321	394
2100	24	97	170	134	207	280	243	316	389

[a]Intensity as the work rate in per cent of the highest work rat that be sustained for 60 minutes.

2.2 Prolonged work capacity

Likewise, the absolute change in (absolute) prolonged work capacity is primarily a function of the intensity and frequency of training or the total amount of work during training (see Table 5). (As with short time work capacity the correlations are the same if the change in work capacity is expressed per kg of body weight or as a percentage of the initial value instead of absolute figures, and these variants are therefore excluded in the following.)

As was the case with the change in short time work capacity the change in prolonged work capacity proved, following multiple regression, to be primarily a function of the intensity and frequency with which the training was conducted (see Table 6). If in addition to intensity and frequency duration or initial work capacity were introduced as a third explaining variable, the coefficient of multiple correlation increased but little (from 0 78 to 0.82 and 0.82 respectively).

In Table 7 the increase in prolonged work capacity for the different training programs has been calculated according to equation 7 in Table 6 and in Table 9 the increase in prolonged work capacity as a function of various combinations of training programs and initial capacity for prolonged work has been calculated according to equation 8 in Table 6

TABLE 9 Predicted absolute change in the capacity for prolonged work (CPW) in kpm x min^{-1} as a function of the intensity (in %) and frequency (times per week) of training and initial CPW (kpm x min^{-1}) when training 60 min per session (eq. no. 8 in Table 6). In order to calculate equivalent values when training 15 or 120 min per session subtract 26 or add 35 kpm x min^{-1} respectively to the given values.

Intensity[a]	50			75			100		
Frequency	1	3	5	1	3	5	1	3	5
Initial CPW									
600	97	194	291	220	317	414	343	440	536
700	79	176	272	202	299	395	325	421	518
800	61	157	254	184	280	377	306	403	500
900	42	139	235	165	262	358	288	385	481
1000	24	120	217	147	243	340	270	366	463
1100	5	102	198	128	225	321	251	348	444
1200	−13	83	180	110	206	303	233	329	426
1300	−32	65	161	91	188	284	214	311	407
1400	−50	46	143	73	169	266	196	292	389

[a]Intensity as the work rate in per cent of the highest work rate that can be sustained for 60 minutes.

3 The relationship between the capacity for prolonged and short time work and the change in this relationship

The relationship between the capacity for prolonged and short time work was highly significant both prior to the training ($r = 0.89$) as well as after 8 weeks of training ($r = 0.95$). The difference in correlation is not significant.

In absolute terms, the average increase in the capacity for short time work was almost as great as the increase in the capacity for prolonged work (256 kpm x min^{-1} and 270 kpm x min^{-1} respectively). A comparison of the figures for the absolute increase in Table 7 shows that no training program had any selective influence on either prolonged or short time work capacity.

In as much as the capacity for short time work is greater than that for prolonged work, an equally great increase for each implies in absolute terms that the relationship between the capacity for prolonged and short time work is changed and also implies a change in slope for the relationship between the logarithms for work time and work rate. The coefficient of regression increased from an average of −5.9 before training to −7.1 after four weeks of training and to −7.4 after the completion of training. The difference in slope before and after training is highly significant (see Table 4).

IX. DISCUSSION

1 Methodological problems

1 1 The mode of expressing work capacity

In this study work capacity is expressed as the highest work rate the individual can sustain for 6 or 90 minutes, respectively From a logical point of view this is not correct, since the maximum work time is a consequence of the work rate and not vice versa. The fact that this method of expression was nevertheless used is due, among other things to the following circumstances. If, after training, each test subject had performed at the work rates which prior to training resulted in work times of 6 and 90 minutes, the work time would in certain cases have been more than doubled or even doubled many times over This was not desirable, since maximum work tasks of widely different duration do not have the same physiological significance. For this reason the work rates were chosen in such a way that the maximum work was approximately 6 or 90 minutes respectively Furthermore, with the use of different work rates one cannot make a comparison between the individual's results before and after training (or between results for different individuals) solely on the basis of work time. However the mode of expressing work capacity introduced by Tornvall (1963) does make possible a comparison of results obtained from tests at different work rates, and it has therefore been used in the present study

1.2 Conversion of maximum work time at a certain work rate into maximum work rate for a set work time

In individual cases the work time of course deviated from 6, or 90 minutes respectively even though the work rate was chosen with these work times in mind. Thus, the highest work rate the test subject should have been able to sustain for 6 or 90 minutes respecitvely must be calculated on the basis of actual work rate and work times. For this purpose, formula (1) and (3), respectively (see section VI.3) were used. The constant b in these formulas is the slope of the straight line, describing the relationship between the logarithmic values for work time and work rate.

In calculating the work capacity one should ideally use the 'true value for the constant b. However this true value is unknown, which means that one has to use an approximate value. In the present study the average value for the constant b was −5 9 −7 1 and −7 4 respectively during the first, second, and third test occasions, while the average regression in the two sets of military conscript material previously reported (Tornvall 1963 Ahlborg 1966) was −5 0 and −5 7 respectively However during these previous studies the bicycle ergometer model AM 361 was used, i.e., the type with which the actual work rate increases during work of long duration (see Table 2). This probably means that the absolute value of the coefficient of regression was actually somewhat greater than 5.0 and 5 7

However as long as the actual work times are close to the reference time (6 or 90 minutes), it hardly matters whether one sets the value of the constant b at –5 0 –6 0 or –7.5 This can easily be checked by a few calculations.

If as in the present study each test subject has been tested at two different work rates in each round of tests, then it is possible to make an individual estimate of the coefficient of regression However this estimate is probably so uncertain that it is of no advantage to use it in calculating work capacity

Comparisons have been made between work capacity calculated according to the following coefficients of regression a) the estimate made on an individual basis at each round of tests b) the average for the group at each round of tests (–5 9 –7 1 and –7 4) c) –5 0 d) –6 0. The results are approximately the same in all four cases (see Table 10), and one can summarize in the following way what has been learned from this comparison and observations made in other studies

1 In bicycle ergometer tests of this type the work rate ought to be adjusted to the individual, in order that actual work time will be close to the reference time.
2. As a rule, in calculating work capacity one ought to use a mean value for the constant b. This mean value can be calculated on the basis of the test material at hand or equivalent material.
3 Even if the actual work time departs considerably from the reference time, it is doubtful whether it is appropriate to use individual values for the constant b in calculating work capacity if these values are based solely upon two tests. It is probably more appropriate to use the mean value for the constant b especially if the test material is relatively homogeneous.

The results presented in this study have all been calculated on the basis of the mean value obtained at each of the three rounds of tests.

TABLE 10 Difference between values of physical work capacity calculated for different b-values (n = 3 x 27)

Employed b-value		$W_{max\ 6\ min}$		$W_{max\ 90\ min}$	
I	II	$\bar{d}$	SD_d	$\bar{d}$	SD_d
–5.0	–6.0	9	24	–3	13
–5.0	$\bar{b}_w$	17	41	–6	22
–5.0	b ind	16	42	–7	21
–6.0	$\bar{b}_w$	7	18	–2	9
–6.0	b ind	6	24	–4	12
$\bar{b}_w$	b ind	–1	20	–2	11

$\bar{b}_w$ weighted mean of slope during each test period

b ind = individual values of slope during each test period

1.3 Reliability in determining work capacity

The methods used in determining the capacity for short time and prolonged work have been proved to be highly reliable (Nordesjö 1974 b). During a test and retest procedure (without intervening training) it was shown that the result at the retest was on the average somewhat higher than the value obtained in the first instance. The average increase was 6 and 52 kpm x min^{-1} for short time and prolonged work capacity respectively (Whether this increase can be said to be due to habituation or to the fact that the first work test involved a certain training—see section IX.1 7—is of no significance in this context.) This means that one underestimates the precision of a single determination if one calculates it according to the commonly used formula.[1] For this reason, a variant of this formula has been used instead.[2] The precision of a single determination of short time and prolonged work capacity calculated in this way is 62 and 47 kpm x min^{-1} respectively

1.4 Intended and actual intensity

According to the test plan, 9 test subjects were to train at 50 %, 9 at 75 %, and 9 at 100 % of the highest work rate the test subject could sustain for 15 60, or 120 minutes—i.e., the average intensity was to be 75 %. In as much as the work capacity increased during training, the intensity diminished, relatively speaking. In order to be able to adjust the work rate during training so that the intensity was once again the intended, the test subject had to carry out a new short time and prolonged work test after four weeks, when the first half of the training was concluded. The average intensity was 78 and 77%, respectively at the beginning of the first and fifth week of training, but it was lower (68 and 72%, respectively) at the end of the fourth and eighth week and the average mean intensity was 73 and 74.5%, respectively However the two work tests carried out after the fourth week cannot be disregarded from the standpoint of training but must be included as two training sessions during which the intensity was 100 % The average mean intensity then turned out to be 75 %. Consequently the obtained intensity for the whole group was rather close to the intended inspite of considerable increases in work capacity in certain cases.

Ideally the work rate should be adjusted every day of training in parallel with improvement in the physical fitness of the test subject. However this is hardly possible with the method and the definition of intensity used in this study If instead intensity is defined as a certain heart rate, then one can allow the heart rate to control the work rate during each training session so that the intensity—i.e., the heart rate—always remains the same. However there are certain disadvantages with this arrangement. The control of heart rate requires either an increased number of test personnel or a sophisticated electronic regulation system. Moreover it was found in the present study that the difference in heart rate between two training sessions, when training at the

1 $\sqrt{\frac{\Sigma d^2}{2n}}$ 2 $\sqrt{\frac{\Sigma (d-\bar{d})^2}{2(n-1)}}$

same work rate and on two consecutive days of training varied between −25.5 and +24.6 beats x min^{-1} ($\bar{x}$ = −0.3 SD = 7.3 beats x min^{-1}).

The sum of differences between the final heart rate during the first and last training session of both rounds of training can be taken as a measure of the decrease in intensity (Table 12). An appreciable decrease in heart rate was registered only for those who trained at 100 % intensity

1.5 Different ways of expressing intensity

In this study the intensity of training has been expressed as a percentage of the highest possible intensity that can be sustained for the duration of the training session. Thus, in absolute terms the intensity 100 % denotes different work rates depending upon whether the training session is 15 60, or 120 minutes long, but this also means that the test subject is exhausted at the end of the training session.

In certain other studies the intensity of training is expressed as the work rate in per cent of the lowest work rate requiring full utilization of the maximum oxygen uptake (= % $\dot{V}_{O_2 max}$). (Work done at this work rate is usually designated as maximal and work done at lower work rates irrespective of the duration of work or the degree of exhaustion as submaximal (Lange Andersen *et al.* 1971). Work done at loads higher than the lowest load which results in maximal oxygen uptake has been designated as supermaximal (Karlsson 1971).)

Defined in this way 100 % intensity always denotes the same absolute work rate of training for a stated test subject, whatever the duration of the training session. On the other hand, the degree of exhaustion is extremely dependent upon the duration of the training session. This is also the case when the intensity is referred to as work rate in percent of W_{max}. The simplest way of measuring intensity is, however to state the absolute work rate of training.

In a few studies (Karvonen *et al.* 1957 Roskamm *et al.* 1966 a and b Kilbom 1971 a, b and c) heart rate has been used as a measure of the intensity of training. Such studies have then reported the difference between (mean) heart rate during training and heart rate at rest. I.e. pulse increase (PI = HR_{work} − HR_{rest}), relative to the pulse increase capability (PIC = HR_{max} − HR_{rest}) i.e. PI/PIC.

Since it is rather difficult to obtain a reliable value of an individual's heart rate at rest, it might, at least in homgeneous groups, be better to exchange individual heart rate at rest for the group mean in the formula above or to express training intensity as heart rate during training *per se* (HR_{work}) or relative to the maximal pulse (HR_{work}/HR_{max}).

1.6 Room temperature

In four cases the room temperature during the tests prior to and after training was 20–21°C and 25–26°C respectively The significance of this is difficult to determine.

Studies concerning the importance of temperature have generally dealt with considerably larger intervals of temperature than those reported here. In a few studies an

session is not significant (Table 5), and does not become significant even if one takes into consideration the intensity and frequency with which the training was conducted (Table 6).

As mentioned earlier the significance of duration has been partially obscured by the fact that the intensity of training has been calculated on the basis of the duration of the training session. If intensity is instead expressed as work rate in per cent of $W_{max\ 6\ min}$ then there does exist a significant partial correlation between the increase in work capacity and the duration of the training session (see Table 11). Moreover this partial correlation is of the same magnitude as the partial correlation between frequency and change in work capacity The partial correlation between duration and increase in prolonged work capacity is greater than that between duration and increase in short time work capacity

Summarizing, one can then assert that all three factors—intensity frequency and duration—are of essential importance for the change in work capacity but that the relative importance in the analysis of regression or correlation is dependent upon the chosen measure of intensity

4 Training and heart rate

Maximum heart rate is in this study defined as the heart rate recorded at the end of work during tests on the capacity for short time work. However this heart rate is 3.5 beats x min^{-1} lower than the final heart rate recorded during the graded work tests to exhaustion which were conducted prior to the first test on short time work capacity This is in agreement with Tornvall (1963) who found a corresponding difference of 5.5 beats x min^{-1}

The training did not lead to any decrease in heart rate at the end of the short time test (Table 3). On the other hand, there was a decrease in heart rate of 9 beats per min when the first and last session of each training round were compared (see Table 12).

In this study an attempt has also been made to illustrate the effect of stating intensity in terms of heart rate during training. An expression of the heart rate during training

TABLE 12. Change in heart rate during the last minute of the training session. Sum of differences *between the first and last session in each training period. Mean for the 3 subjects who trained 1 3* and 5 times per week.

Intensity of training, %[a]	Duration of training session, min		
	15	60	120
50	+14	−9	−2
75	+5	−4	−11
100	−18	−19	−29

[a]Intensity as work rate in per cent of the highest work rate that can be sustained during the training session.

(HR_{work}) for each individual is obtained by adding together the integrated heart rates from all training sessions and from the two middle tests and then dividing by the total time. The training heart rate has then been set in relation to the maximum heart rate (HR_{max}) and the heart rate at rest (HR_{rest}) in order to obtain the four measures of intensity described previously (see section IX.1.5). These four measures of intensity based upon heart rate are highly intercorrelated and therefore only HR_{work}/HR_{max}, i.e. an expression of the intensity of training which is not based upon heart rate at rest, is compared with other variables.

There is a small and insignificant correlation between HR_{work}/HR_{max} and change in work capacity ($r_{CSW} = 0.32$ and $r_{CPW} = 0.28$). Following multiple regression between change in work capacity and the measure of intensity given above, the frequency and duration of training the partial correlation between change in work capacity and intensity is highly significant (see Table 11). The regression equations are for change in short time work capacity (Δ CSW) $= -651 + 9.00\ I + 37.7\ F + 1.82\ D$ and for change in prolonged work capacity (Δ CPW) $= -677 + 9.11\ I + 43.9\ F + 2.00\ D$ where I = HR_{work}/HR_{max} *in percent, F = number of training sessions per week, and D = duration of training session in minutes.*

The HR_{work}/HR_{max} registered at different times during the training period has been graphed in Fig. 4. The curves represent the mean value for those training 1, 3 or 5 times a week at the same intensity (per cent of the highest possible work rate during a

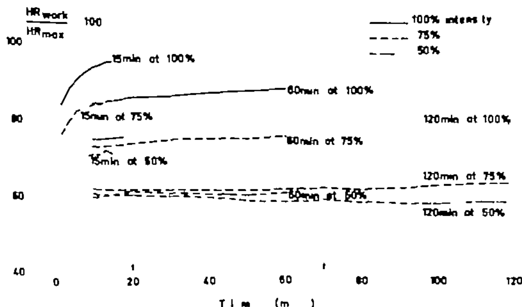


Fig. 4. Heart rate at set time during the training session (HR_{work} = sum of heart rate at set time of all training sessions/no. of training sessions) as percentage of the heart rate at end of test on short time work capacity (HR_{max} = mean of final heart rate at the first and last test of short time capacity). The *curves represent a mean for those who trained 1, 3 or 5 times a week at the same intensity* (% of the highest work rate that can be sustained during set time) and duration.

set time) and duration Fig. 4 shows that certain training programs were the same with regard to HR_{work}/HR_{max}, namely

15 min x 75 % and 60 min x 100 %
60 min x 75 % and 120 min x 100 %
60 min x 50 % and 120 min x 75 %

Table 13 shows that the increase in the capacity for short time and prolonged work for equal values of HR_{work}/HR_{max} is greater when the duration of the training session is longer

TABLE 13 Increase in physical work capacity as a result of training of varying duration but at approximately equivalent heart rates. Mean of the integrated heart rate after 10 min expressed as a percentage of maximal heart rate, work capacity expressed in kpm x min^{-1} Mean values for subjects who trained 1 3 or 5 times week.

Training program intensity[a]	duration of session, min	Heart rate %	Change in CSW	CPW
75	15	84	213	182
100	60	86	340	460
75	60	74	308	256
100	120	77	489	428
50	60	60	134	163
75	120	62	229	248

CSW = capacity for short work
CPW capacity for prolonged work

[a] intensity as the work rate in per cent of the highest work rate that can be sustained during the training session.

5 The relationship between increases in the capacity for short and prolonged work

The absolute increase in the capacity for short time and prolonged work is of the same magnitude (on the average 256 respectively 270 kpm x min^{-1}). The fact that the capacity for short time work is numerically greater than that for prolonged work means that the relative increases for both are of different magnitudes (16.5 and 27 4 % respectively) and that the coefficient of regression in the relationship log time-log work rate increases numerically from −5 9 to −7 4

The covariation between the increase in the capacity for short time and prolonged work is highly significant (r = 0 79). Thus, none of the training programs have selectively affected the capacity for short time and prolonged work.

Normally the capacity for short time and prolonged work covariate with each other and both are intimately connected with body dimensions and maximum oxygen uptake (Tornvall 1963 Nordesjö 1974 a). Under normal conditions training can only give

marginal effects. In this context it is of little interest that one can vary the glycogen content of the muscles by means of prolonged work in combination with different diets and thereby selectively influence the capacity for prolonged work (Ahlborg *et al.* 1967 Hermansen *et al.* 1967).

6 Prediction of work capacity

As shown by Table 5 it is possible with a certain degree of certainty to predict the capacity for short time and prolonged work, even on the basis of such simple measurements as information on smoking habits and physical activity

However an estimation of work capacity on the basis of the best combination of measurements for body dimensions and information on activities—i.e., heart volume and recreational physical activity—has a mean error (s) of 150 and 107 kpm x min^{-1} respectively *for short time and prolonged work capacity*

Thus, it is only by conducting a work test that one can reduce the error of estimation to a reasonable magnitude, i.e., about 50 kpm x min^{-1}

7 The relationship between the amount of training and the effect of training

Siegel *et al* (1970) proposed that the relationship between the amount of training and the increase in maximal oxygen uptake is S-shaped. In other words, there is practically no improvement in work capacity if the amount of training falls below a certain threshold. Furthermore, the increase in work capacity rapidly diminishes when the amount of training exceeds a certain limit. This hypothesis was based in part upon certain training studies (Karvonen *et al.* 1957 Hollman and Venrath 1963 Roskamm *1967) in which it was found that work capacity increased only when the increase in* heart rate during training exceeded 60 % of the pulse increase capability ($HR_{max} - HR_{rest}$) i.e. about 130 beats x min^{-1} Furthermore, Mann *et al.* (1969) found that those test subjects who trained during 65 to 103 of projected 130 training sessions produced results of the same magnitude as those who trained 104 to 130 times.

The results obtained by Davies and Knibbs (1971) might also be explained by the existence of a threshold level, in as much as the test subjects in their study who trained at 50 % or less of $\dot{V}o_{2\,max}$ did not increase their maximum oxygen uptake.

On the other hand, Shephard (1968) has registered an increase of estimated maximum oxygen uptake at as low a training intensity as 39 % of estimated $\dot{V}o_{2\,max}$. Kilbom (1971 c) obtained training effects for women in the age group 19—48 years even when intensity was as low as 44 % of $\dot{V}o_{2\,max}$

If according to principles refered to previously the training intensities used in the present study are converted into percentage figures of estimated $\dot{V}o_{2\,max}$ it will be seen that they correspond to 36—94 % of estimated $\dot{V}o_{2\,max}$. Thus, the lowest intensities are

then close to the 35 % level of $Vo_{2, max}$ which an individual can sustain during an eight hour workday (including lunch and breaks) without becoming appreciably tired (Micheal *et al.* 1961 Åstrand, I. 1967 Bonjer 1968), a level which probably would not have any training effect. Inspite of this, the three test subjects who trained at 50 % of the possible intensity in 120 minutes i.e. at 36–37 % of estimated $Vo_{2, max}$ (41 4 % of pulse increase capability) achieved an increase of 88–157 kpm x min^{-1} (7–12 %) in the capacity for short time work ($W_{max\ 6\ min}$) and an increase of 117–302 kpm x min^{-1} (12–41 %) in the capacity for prolonged work ($W_{max\ 30\ min}$).

The contradictory reports on the existence of a threshold might possibly be explained by the fact that one seldom has complete and wholly reliable information on the activity patterns of the test subjects before and during the training studies. This fact leaves open the possibility that the test subject "compensates" for the programmed training by reducing his normal physical activity If the test subject takes advantage of this possibility the result can give the impression that the threshold is higher than it actually is or that there is a threshold even though a threshold does not really exist. We do not have unobjectionable proof for the existence of a threshold. Until such is found perhaps the following, alternative hypothesis will serve just as well as a foundation for discussion and continued research all increase of physical activity normally leads to an improvement in the capacity for physical work, provided that the physical activity does not already be at a very high level.

Even if one can challenge the first part of Siegel s hypothesis in which he assumes the existence of a threshold, there is still much which bespeaks that his assumption of rapidly diminishing returns, once the training has exceeded a critical level, is correct. This would imply that, even if there does not exist any threshold, the effect of training is not a linear function of the intensity frequency and duration of training.

Despite the fact that the data in the present study have been processed by means of linear regression analysis, most of the variation regarding the increase in the capacity for short time and prolonged work could be explained by the frequency duration, and intensity of the training (R = 0.74 and 0.83 respectively). In order to check the possibility that a non-linear analysis might better explain the observed training effects, the residual variance for the increase in prolonged work capacity has been calculated on the basis of an approximative method supplied by Ezekiel and Fox (1959). The residual variance obtained in this manner does not differ significantly from that obtained by linear analysis. This in no way disproves Sigel's hypotehsis The present study was not designed to test this hypotehsis. However the reported equations should not be used for extrapolations outside of the observed range.

8. Comparison between the present study the study by Shephard, and the study by Davies and Knibbs

Table 14 summarizes the test subjects characteristics, experimental procedure and the result of the three studies. As a means of expediating comparisons between the three

TABLE 14 Summary of three studies wherein intensity frequency and duration of training have been varied in a systematic manner

	Shepard (1968)	Davies & Knibbs (1971)	Nordesjö (1974)
Subjects	non-athletic students	students of physical education	non-athletic students
Sex	not mentioned	males	males
Age	19–41	18–38	20–26
Initial $\dot{V}_{O_2}$ max, lit x min^{-1}	3.30	3.35	3.34
ml x kg^{-1} x min^{-1}	43 (SD 5.8)[a]	48 (SD 4 4)	50 (SD 5 7)[b]
Training			
Type	treadmill	bicycle ergometer	bicycle ergometer
Intensity % of $\dot{V}_{O_2}$ max	39 75 and 96[c]	30 50 and 80	36–94[d]
Frequency times per week	1 3 and 5	1 3 and 5	1 3 and 5
Duration of each session, min	5 10 and 20	5 10 and 20	15 60 and 120
Duration of training, weeks	3 4 and 6[e]	8	8
Result			
Estimated mean increase of			
V_{O_2} max, lit x min^{-1}	0.29 (9%)[f]		0.43 (13%)
ml x kg^{-1} x min^{-1}		0.37 (<1%)	
Explaining variables in multiple regression r analysis of covariance and their significance			
Initial V_{O_2} max	***	not included	not included
Intensity % of $\dot{V}_{O_2}$ max		*[g]	**
Frequency	*		
Duration		[g]	*

Estimated from submaximal work test (steptest)

[b]Estimated from maximal work test (W_{max} 6 min)

[c]In Shepherd s study work rate was not adapted t the individual' maximal oxygen uptake. On the contrary three fixed combinations of treadmill speed and slop were used which required 39 75 and 96 % of the subject mean estimated $\dot{V}_{O_2}$ max

[d]In the present study th intensity of training was 50 75 or 100% of the highest work rate the subject could sustain for set duration (15 60 or 120 min) and consequently the intensity varies between 36 and 94 % if it is expressed as per cent of V_{O} max. If required the work rate was adjusted after 4 weeks of training.

[e]The subjects who trained once week trained for 6 weeks, those who trained 3 times a week trained 4 weeks and those who trained 5 times week trained 3 weeks. No reasons for this are stated and the means f elucidating the importance f frequency are rendered more difficult by the described procedure

[f]Estimated increase in $\dot{V}_{O_2}$ max if the subjects train 3 times week at 75 % of $\dot{V}_{O_2}$

[g]Analysis of covariance

studies, the data in the present study has been converted into litres of oxygen uptake per minute. However it should be kept in mind that whichever of the three variables—intensity frequency and duration—best explains the increase in work capacity largely depends upon the extent to which they have been varied. There does not exist any common measure for the variation of intensity frequency and duration which would make it possible to make the variation of the three variables wholly comparable.

Shephard (1968) found that the absolute increase in estimated aerobic capacity was chiefly due to the individual's estimated, initial maximal oxygen uptake ($r = 0.59$). Following multiple regression a significant relationship ($p < 0.05$) could also be found between the increase in estimated aerobic capacity and the intensity and frequency of training.

The duration of the training session was, however not of significant importance. A certain training effect was obtained even when the test subject trained at low intensity (39% of estimated $\dot{V}o_{2\,max}$), but the most effective training program was a combination of the highest intensity frequency and duration used in the study The aerobic capacity of an individual with an initial oxygen uptake of 3.31 x min^{-1} and weight of 76 kg can be calculated to increase 0.29 l x min^{-1} (9%), if he trains 3 times a week for 4 weeks at an intensity which equals 75% of estimated $\dot{V}o_{2\,max}$, i.e. one of the middle programs.

Davies and Knibbs (1971) found that intensity was the most important factor for the increase of maximum oxygen uptake. Those who trained at 30 % or 50 % changed very little or not at all independent of the frequency and duration of training, but according to the figures for those who trained at 80 %, the extent of the change correlated both with the duration and frequency of the training. Even at the highest intensity (80 % of $\dot{V}o_{2\,max}$) and greatest duration (20 min) the increase in aerobic capacity was very moderate from minus 1 to plus 9 ml x kg^{-1} x min^{-1}(1–19 %). As a whole, the group's aerobic capacity increased by 0.37 ml x kg^{-1} x min^{-1} (< 1 %).

In the present study the change in work capacity was found to be chiefly related to the intensity of training but also to its frequency and duration (see Table 14).

The most obvious difference in the results obtained by the three studies is the almost total lack of increase in aerobic capacity in the study of Davies and Knibbs. The test subjects in the last mentioned study were students of physical education, i.e., a group which probably is more physically active than the other two groups studied, even though the maximal oxygen uptake capacity is the same in the present study Davies and Knibbs have also had the subjects exercising daily for 14 days immediately prior to the determination of the initial value for maximal oxygen uptake. Unfortunately further particulars of this pretraining have not been given. If such an extensive program of physical activity is not to influence work capacity this presupposes either that the subject is already rather well-trained or that the work rate is extremely low

In the present study 2/3 of the training sessions were of very long duration (60 and 120 min), and as shown previously the duration of the training session is of significance (see section IX.3 4 and Table 11). But even the 9 subjects who trained for 15 minutes each session increased their estimated maximal oxygen uptake more than what was

reported in the other two studies where the training sessions were 5 10 or 20 min long.

Moreover in the present study the work rates for training were adjusted after 4 weeks of training. This probably led to a greater increase in work capacity than that which would have resulted if the work rates had not been raised. But even after 4 weeks of training the average increase in estimated maximum oxygen uptake capacity was of the same magnitude (0.27 l x min^{-1} 8 %) or greater than that reported in the other two studies. This is also the case for the groups which trained only 15 minutes each training session (0.31 l x min^{-1} 9 %).

The number of training weeks in Shephard's study was not equal for those who trained 1 3 or 5 times a week. As far as can be determined, Shephard has not taken this into consideration when processing his data. For this reason he may have under or overestimated the importance of the frequency of training for the increase in work capacity

In all three studies it appears as if the intensity of training is of greater importance for the increase in work capacity than the frequency and duration of training. However the importance of duration would need to be illustrated further by means of ex periments in which the length of the training sessions varied over a greater interval of time, for example 5 to 120 minutes, but in which the frequency and intensity of the training was kept constant.

9 Regarding the suitability of expressing the increase of work capacity in percentage terms

When different individuals train according to the same training program, it usually happens that those who initially had high work capacity on an average achieve less of an increase in work capacity than those who initially had a low work capacity (Saltin *et al.* 1968). As previously pointed out (see section IX.3 1), this is probably more due to the fact that there is a greater number of well-trained individuals in the group with high work capacity as compared with the group with low work capacity than that initial work capacity *per se* is of any importance. The expression "the same training program" used above, implies that the intensities of the training are adjusted accor ding to the individual and that, from a subjective point of view they are equal. When it comes to training work rates which, in an absolute sense, are equal, it is probable that both the initial degree of training and the initial absolute work capacity are of impor tance for the result.)

The suitability of expressing change in work capacity as a percentage of the initial value can be questioned, *inter alia* for the following reasons

1 In the training of individuals with varying initial work capacity the increase is not directly proportional (but rather inversely proportional) to the initial work capacity
2. Even if the absolute increase is equally great for all the test subjects, an obvious relationship is obtained between the relative increase and the initial level (on the premise that this varies).

3 Statements such as 'the work capacity increased by 10 per cent' are rather meaningless if one does not also state the measure used for work capacity as well as the initial level. It can hardly be said that all conceivable measures of work capacity increase percentually to the same extent during training. But once the increase is given in percentage terms, it is easy to be tempted into making statements which presuppose that the increase for different variables mentioned in various studies are directly comparable when expressed in percentage terms.

10 The coefficient of regression in the relationship between log work time and log work rate

10.1 Initial slope as a function of measures of physical dimensions and activity
The initial slope has weak but insignificant relationships with heart volume, smoking, occupational and recreational physical activity. The multiple correlation is 0.43 ($p > 0.05$) with heart volume, smoking, and occupational and physical activity as explaining variables and 0.46 ($p > 0.05$) with heart volume, smoking, and recreational physical activity as explaining variables. The partial correlations between initial slope and one of the other variables are not significant. The relationships are so weak that one must draw the following conclusion: It is impossible, on the basis of measurements of dimensions or activities, to determine in advance and with any great deal of certainty whether a person has a high or low capacity for prolonged work, relative to the capacity for short time work.

The result might, however, have been different if the coefficient of regression had been based upon more than two pairs of values.

10.2 Change in slope as a function of training
Training leads to a significant increase in slope, and there is a weak relationship ($R = 0.45$, $p > 0.05$) between the numerical increase in slope and the intensity, frequency and duration of training. Partial correlations are not significant. Thus, it was impossible to show the existence of any relationship between the change in the coefficient of regression and the length of the training sessions. Even though 1/3 of the training sessions were extremely long this did not lead to any selective increase in the capacity for prolonged work.

11 The test subjects' subjective experiences

In connection with the training, notations were made of the test subjects' experience of the undertaking. This can be summarized as follows:

1 In training at the 100 % level 5 times a week the test subjects had constant muscular ache, and their muscles were always tender and indurated. Although training at the

100 % level 3 times a week was experienced as strenuous, it did not cause constant muscular ache.

2. Training at the 50 % level for 60 or 120 minutes was felt to be too easy
3. Training periods of 2 hours were too monotonous. Sessions of 15 and 60 minutes duration were more acceptable.

12. Choice of training program

A reasonable goal of training for the average 20 to 30 year old might possibly be an increase of about 250 kpm x min^{-1} in the capacity for short time and prolonged work. According to Table 7 an increase of 250 kpm x min^{-1} after two months of training can be achieved in several different ways, for example

1 time per week x 15 min x 100 %
3 times per week x 60 min x 75 % and
5 times per week x 120 min x 50 %

The test subjects subjective experiences from the training indicate that, given a free choice among these three programs, they would prefer to train 3 times a week for 60 minutes at 75 % intensity. However different individuals have different preferences. For example, some are unwilling to exert themselves (train at more than 50 %), while others enjoy exerting themselves almost to the point of utter exhaustion and do not consider the high training intensity as something negative. Then there are certain individuals who feel that they are more pressed for time than other people, etc. Thus, in spite of a common goal of training, different individuals can choose different ways of reaching this goal on the basis of varying preferences.

Figure 5 illustrates the effect of several different training programs on the capacity for short time work as based on the premise that the individual is initially rather untrained. The three continuous lines, which are based on equation 4 in Table 6 show the effect of training 3 times a week for 60 minutes at at relative intensity of 50 75 and 100 % respectively. The increase is approximately the same, whatever the absolute value of the initial work capacity.

The dashed line represents the amount of increase in work capacity achieved by 197 military conscripts during the first part of their basic instruction, one which involves a relatively high degree of physical training (Linroth and Nordesjö 1968). The training was carried out in groups of about 30 persons, and within each group there was a rather wide range of initial physical fitness and work capacity. The relatively steep slope of this line is most likely due to the following conditions. When training is conducted on a group basis and the work capacity varies within each group, all of the members of the group will be training at approximately the same absolute intensity but at a varying amount of relative intensity. Those with the lowest degree of work capacity train at high relative intensity. Thus, they achieve a greater increase than those have a high work capacity and who consequently train at a low relative Furthermore, individuals with high work capacity are on the average

3 Statements such as 'the work capacity increased by 10 per cent' are rather meaningless if one does not also state the measure used for work capacity as well as the initial level. It can hardly be said that all conceivable measures of work capacity increase percentually to the same extent during training. But once the increase is given in percentage terms, it is easy to be tempted into making statements which presuppose that the increase for different variables mentioned in various studies are directly comparable when expressed in percentage terms.

10 The coefficient of regression in the relationship between log work time and log work rate

10.1 Initial slope as a function of measures of physical dimensions and activity
The initial slope has weak but insignificant relationships with heart volume, smoking, occupational and recreational physical activity The multiple correlation is 0.43 ($p > 0.05$) with heart volume, smoking, and occupational and physical activity as explaining variables and 0 46 ($p > 0\ 05$) with heart volume, smoking, and recreational physical activity as explaining variables. The partial correlations between initial slope and one of the other variables are not significant. The relationships are so weak that one must draw the following conclusion It is impossible, on the basis of measurements of dimensions or activities, to determine in advance and with any great deal of certainty whether a person has a high or low capacity for prolonged work, relative to the capacity for short time work.

The result might, however have been different if the coefficient of regression had been based upon more than two pairs of values.

10.2 Change in slope as a function of training
Training leads to a significant increase in slope, and there is a weak relationship ($R = 0.45$ $p > 0.05$) between the numerical increase in slope and the intensity frequency and duration of training. Partial correlations are not significant. Thus, it was impossible to show the existence of any relationship between the change in the coefficient of regression and the length of the training sessions. Even though 1/3 of the training sessions were extremely long this did not lead to any selective increase in the capacity for prolonged work

11 The test subjects subjective experiences

In connection with the training, notations were made of the test subjects experience of the undertaking. This can be summarized as follows

1 In training at the 100 % level 5 times a week the test subjects had constant muscular ache, and their muscles were always tender and indurated. Although training at the

the mechanical efficiency of walking is 22 %. Even in this case the sharp slope of the curve can be explained with reference to varying relative intensity

EKBLOM, B P.-O ÅSTRAND, B SALTIN, J STENBERG and B. WALLSTRÖM, Effect of training on circulatory response to exercise. *J appl. Physiol.* 1968. *24* 518–528
EZEKIEL, M and K.A. FOX, Methods of correlation and regression analysis. Wiley N Y 1959
HAISMAN M.F *A review of physical fitness tests with particular reference to the maximal oxygen intake.* Personnel research establishment of the British Army Research memorandum P/3 February 1967
HERMANSEN, L., E. HULTMAN and B. SALTIN, Muscle glycogen during prolonged severe exercise. *Acta physiol scand.* 1967 *71* 129–139
HOLLMANN W., *Höchst und Dauerleistungsfähigkeit des Sportlers.* Johann Ambrosius Barth, München. 1963
HOLLMANN, W G HERKENRATH, S. GRÜNEWALD, H. BUDINGER, U JONATH, H. RÜSSMANN and D HAIN, *Untersuchungen über Möglichkeiten zur Steigerung des körperlichen* Leistungsvermögens von Rekruten. *Sportarzt und Sportmedizin* 1966 *12* 582–592.
HOLLMANN, W and H. VENRATH, Die Beeinflussung von Herzgrösse, maximaler O_2 Aufnahme und Ausdauergrenze durch ein Ausdauertraining mittler und hoher Intensität. *Der Sportarzt* 1963 *14* 189
HOLMGREN A., Optimum physical performance capacity in adults. *Wld Hlth Org techn. Rep. Ser* No. 436 1964
HOLMGREN A. and K. H. MATTSON A new ergometer with constant load at varying pedalling rate. *Scand. J clin. Lab. Invest.* 1954 *6* 137–140.
KARLSSON J Lactate and phosphagen concentrations in working muscle of man. *Acta Physiol. Scand.* 1971 *81* Suppl 358 17
KARRASCH. K. and E.A. MÜLLER, Das Verhalten der Pulsfrequenz in der Erholungsperiode nach körperlicher Arbeit. *Arbeitsphysiologie* 1951 *14* 369–382.
KARVONEN MJ E. KENTALA and O MUSTALA, The effects of training on heart rate. A "longitudinal" study *Ann. med. exp. Fenn.* 1957 *35* 307–315.
KILBOM, Å., Physical training in women. *Scand. J clin. Lab. Invest* 1971 a. *28* Suppl. 119
KILBOM, Å., Physical training with submaximal intensities in women. I. Reaction to exercise and orthostasis. *Scand. J clin. Lab. Invest 1971* b. *28 141–161*
KILBOM, Å., Effect on women of physical training with low intensities. *Scand. J clin. Lab. Invest.* 1971 c. *28* 345–352.
KILBOM, Å., L.H. HARTLEY B SALTIN J BJURE, G GRIMBY and I ÅSTRAND Physical training in sedentary middle-aged and older men. I. Medical evaluation. *Scand. J clin. Lab. Invest* 1969 *24* 315–323
KJELLBERG, S.R., H. LÖNROTH and U RUDHE, The effect of various factors on the roentgenological determination of the cardiac volume. *Acta radiol* 1951 35 413–427
KJELLBERG, S.R., U RUDHE and T SJÖSTRAND, The relation of the cardiac volume to the weight and surface area of the body the blood volume and the physical capacity for work. *Acta radiol.* 1949 *31* 113–122.
KLAUSEN K D.B DILL, E.E. PHILLIPS, JR. and D MCGREGOR, Metabolic reactions to work in the desert. *J Appl. Physiol.* 1967 *22* 292–296.
KNEHR, C.A., D.B. DILL and W NEWFELD Training and its effects on man at rest and at work. *Amer J Physiol.* 1942 *136.* 148–156.
LANGE ANDERSEN, K., R.J SHEPHARD, H DENOLIN E. VARNAUSKAS and R. MASIRONI, *Fundamentals of exercise testing* Wld Hlth Org., Geneva. 1971
LINROTH, K., *Medicinska aspekter på ett nytt system för inskrivning av värnpliktiga. Läkartidningen* 1969 *66* 881–892.
LINROTH, K. and L.-O NORDESJÖ, Medicinsk försöksverksamhet. Rapport avseende befattningsanalysarbetet. *1968 års ÖB-rapport"* Kap. 16. (Report to the Commander in Chief 1968). Värnpliktsverket, Stockholm 1968.
MANN, G V., H.L. GARRETT A. FARHI, H. MURRAY and F T BILLINGS, Exercise to prevent coronary heart disease. *Amer J Med.* 1969 *46* 12–27

MICHAEL, E.D K.E. HUTTON and S.M HORVATH Caridorespiratory responses during prolonged exercise. *J appl Physiol.* 1961 *16* 997–1000.

MÜLLER, E.A., Ein Leistungs Pulsindex als Mass der Leistungsfähigkeit. *Arbeitsphysiologie.* 1950. *14* 278–284

MÜLLER, E.A., Wirkungsgrad und Leistungsfähigkeit bei Arbeit mit den Wadenmuskeln. *Int. Z. angew Physiol.* 1955 *16* 25–34

MÜLLER, E.A. *Die Messung der körperlichen Leistungsfähigkeit mit einem einzigen Prüfverfahren.* Forschungsberichten Landes Nordrhein-Westfalen Nr 1031 Westdeutscher Verlag, Köln. 1961

NORDESJÖ, L.-O A comparison between the Tornvall maximal ergometer test, submaximal ergometer tests and maximal oxygen uptake. *Försvarsmedicin* 1974 a. *10* 3–10.

NORDESJÖ, L.-O Estimation of the maximal work rate sustainable for six minutes using a single level load or stepwise increasing loads. *Upp. J Clin. Sc.* 1974 b. *79* 45–50.

PASSMORE, R. and J V G.A. DURNIN Human energy expenditure. *Physiol. Rev* 1955 *35* 801–840.

ROBINSON, S Physiological adjustments to heat. In *Physiology of temperature regulation and the science of clothing* Newburgh, L.H. (ed.). Saunders, Philadelphia. 1949

ROBINSON, S. and P.M. HARMON Effects of training and of gelatin upon certain factors which limit muscular work. *Amer J Physiol.* 1941 *133* 161–

ROHMERT W *Untersuchung über Muskelermüdung und Arbeitsgestaltung* Beuth-Vertrieb GmbH, Berlin. 1962.

RONGE, H XVI. Fysiska miljöfaktorer A. Klimat och belysning. In *Handbok i ergonomi.* Luthman, G U Åberg and N Lundgren (eds.). Almqvist & Wiksell, Stockholm. 1966.

ROSKAMM, H., Optimum patterns of exercise for healthy adults. *Canad. Med. Ass. J* 1967 *96* 895–900.

ROSKAMM, H., N BRANDTS and H. REINDELL, Zur Trainierbarkeit der Herz und Kreislaufleistungsfähigkeit in Abhängigkeit von Alter und Geschlecht. *Cardiologia* 1966. *48* 441–460.

ROSKAMM, H. and C. CLASING Die Abhängigkeit des Trainingseffektes von der Trainingsart. *Sportarzt und Sportmedizin* 1967 *18* 16–25

ROSKAMM, H., KÖNIG, W GÜTTEL, H. WEIDEMANN, R. THOMAE, E. LÖHNE, G BLÜMCHEN, A FRIEDRICHSEN and H REINDELL, Systematische Trainingsversuche auf dem Ergometer bei 80 Rekruten. *Sportarzt und Sportmedizin 1966 17* 569–581

ROWELL, L.B *Factors affecting the prediction of maximal oxygen intake from measurements made during submaximal work with observations related to factors, which may limit maximal oxygen intake* (Thesis). University of Minnesota, Minneapolis. 1962.

ROWELL, L.B J.R. BLACKMON R.H. MARTIN, J.A. MAZZARELLA and R.A. BRUCE, Hepatic clearance of indocyanine green in man under thermal and exercise stresses. *J appl. Physiol.* 1965 *20* 384–394

SALTIN B G BLOMQVIST J.H. MITCHELL, R.L. JOHNSON JR, K. WILDENTHAL and C.B CHAPMAN, Response to exercise after bedrest and training. *Circulation* 1968. *38* Suppl. VII

SALTIN, B., A.P GAGGE, U BERGH and J.A.J STOLWIJK, Body temperature and sweating during exhaustive exercise. *J appl. Physiol.* 1972. *32* 635–643

SALTIN, B and G GRIMBY Physiological analyses of middle-aged and old former athletes. *Circulation* 1968. *38* 1104–1115

SALTIN B., L.H. HARTLEY Å. KILBOM and I. ÅSTRAND, Physical training in sedentary middle-aged and older men. II. Oxygen uptake, heart rate, and blood lactate concentration at submaximal and maximal exercise. *Scand. J clin. Lab. Invest.* 1969 *24* 323–334.

SHEPHARD, R.J The development of cardio-respiratory fitness. *Medical Services J* 1965 *21* 533–544

SHEPHARD, R.J Intensity duration and frequency of exercise as determinants of the response to a training regime. *Int. Z. angew Physiol.* 1968. *26* 272–278.

SHEPHARD, R.J Learning, habituation and training. *Int. Z. angew. Physiol.* 1970. *28* 38—48.
SIEGEL, W., G. BLOMQVIST and J.H. MITCHELL, Effects of a quantitated physical training program on middle-aged sedentary men. *Circulation* 1970. *41* 19—29
SNEDECOR, G.W. and W.G. COCHRAN. *Statistical methods.* The Iowa State University Press, Ames. 1967
TAYLOR, H.L., A. HENSCHEL, J. BROZEK and A. KEYS, Effects of bed rest on cardiovascular function and work performance. *J. appl. Physiol.* 1949 *2* 223—239
TORNVALL, G., Assessment of physical capacilities. *Acta physiol. scand.* 1963 *58* Suppl. 201
WILLIAMS, C.G. G.A.G. BREDELL, C.H. WYNDHAM, N.B. STRYDOM, J.F. MORRISON, J. PETER, R.W. FLEMING and J.S. WARD, Circulatory and metabolic reactions to work in heat. *J. appl. Physiol.* 1962. *17* 625—638.

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 406

STUDIES ON FIBRE SIZE IN DEVELOPING SCIATIC NERVE AND SPINAL ROOTS IN NORMAL UNDERNOURISHED AND REHABILITATED RATS

by

ANDERS SIMA

Göteborg 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 406

From the Neuropathological Laboratory Institute
of Pathology University of Göteborg, Sweden

STUDIES ON FIBRE SIZE IN DEVELOPING SCIATIC NERVE AND SPINAL ROOTS IN NORMAL UNDERNOURISHED AND REHABILITATED RATS

by

ANDERS SIMA

Göteborg 1974

CONTENTS

I Introduction 5

II Material and methods 7

A. Animal material 7
1 Animals 7
2. Animal environment 7
3 Diet 7
4 Undernutrition 7
5 Nutritional rehabilitation 8
B. Histotechnical procedure 8
1 Histological technique 8
2. Methodological considerations 8
3 Fibre counting technique 12
C. Fibre growth velocity 13
D Statistical methods and presentation of the material 13

III Results 14

A. Sciatic nerve 14
1 Calibre spectrum during normal postnatal development in control rats 14
2. Calibre spectrum during postnatal development in undernourished rats 14
3 Effect of postnatal nutritional rehabilitation on calibre spectrum 18
4 Comparison of the calibre spectrum of normal, undernourished, and rehabilitated rats 18
5 Observations on axonal diameters in normal and undernourished rats 19
B Spinal roots 19
1 Calibre spectrum of ventral root (LV) during normal postnatal development in control rats 19
2. Calibre spectrum of dorsal root (LV) during normal postnatal development in control rats 21
3 Calibre spectrum of ventral root (LV) in undernourished rats during postnatal development 21

4 Calibre spectrum of dorsal root (LV) in undernourished rats during postnatal development 26
5 Comparison of calibre spectrum of normal and undernourished rats 26
6 Effect of postnatal nutritional rehabilitation on calibre spectrum of ventral root (LV) 30
7 Effect of postnatal nutritional rehabilitation on calibre spectrum of dorsal root (LV) 30
8. Comments 30

IV General discussion 32

V Summary 36

VI References 37

VII Tables 40

1 INTRODUCTION

In recent years the effect of early undernutrition on the developing nervous system has attracted much attention. In particular the interest has been focused on the central nervous system and the possible causal relationship between undernutrition and the genesis of mental retardation.

As revealed by numerous animal experiments and a limited number of observations on humans, the most common forms of undernutrition i.e. calorie deficiencies and protein deficiencies (cf Mc Cance and Widdowson, 1968) may affect the physical development of the nervous system resulting in various kinds of deficits and distortions (Dobbing and Smart, 1973). These deficits and distortions have mostly been detected by nonhistological methods e.g. estimation of brain weight, determinations of the number of brain cells in tissue samples (Winick and Noble 1965 Winick, 1968 1969 Winick and Rosso, 1969) and determinations of myelin lipids, mainly cholesterol indicating the degree of myelination of cerebral white matter (Dobbing, 1964).

Platt and Stewart (1969) reported observations on spinal cords of young dogs showing that chromatolysis of the motor cells of the anterior horns and an increase in perineuronal oligodendrocytes are prominent but probably reversible phenomena caused by a low amount of protein in the diet.

The effect of early postnatal undernutrition on the growth and chemical maturation of single spinal cord motor neurons of the rat have been studied by quantitative cytochemical methods by Haltia (1970) and Haltia and Sourander (1970 a, b) In these studies it was shown that undernutrition during the first month of life markedly reduces the cytoplasmic RNA content and dry mass after lipid extraction The reduced values both for RNA and dry mass, the latter corresponding to the bulk of proteins, indicate an impaired protein metabolism in the anterior horn motor neurons.

Hitherto only a very limited number of investigations have been published on *the effects of protein calorie deficiencies on the developing peripheral nervous* system (Clos and Legrand, 1970 Hedley Whyte and Meuser 1971 Hedley Whyte 1973) These authors have shown ultrastructurally that nutritional deprivation during early postnatal life causes a decreased number of myelin lamellae in the nerve fibres of the rat sciatic nerve The two latter authors have also demonstrated that there is a decreased axonal circumference, indicating an impaired axonal growth.

Ever since Erlanger and Gasser (1937) made their fundamental discovery by using the cathode ray tube that nerve fibres conduct their impulses at different

4 Calibre spectrum of dorsal root (LV) in undernourished rats during postnatal development 26
5 Comparison of calibre spectrum of normal and undernourished rats 26
6 Effect of postnatal nutritional rehabilitation on calibre spectrum of ventral root (LV) 30
7 Effect of postnatal nutritional rehabilitation on calibre spectrum of dorsal root (LV) 30
8 Comments 30

IV General discussion 32

V Summary 36

VI References 37

VII Tables 40

II MATERIAL AND METHODS

A. Animal material

1 Animals. Virgin rats of the Sprague Dawley strain 3 months of age were mated with males of varying ages up to 8 months. The time of fertilization was determined by vaginal smears twice daily All litters used were adjusted to 8 pups at time of birth. Only male rats were used in this study Control and undernourished rats ranging from 5 days to 180 days of age were investigated As growth is no continuous process, the material had to be divided into several age-groups. Therefore control as well as undernourished rats were examined at 12 different ages. Each age-group consisted of at least 2 animals. Altogether 114 animals were investigated.

2 Animal environment. All control rats and those of the various experimental series were kept in plastic cages at a temperature of 25°C and in a relative humidity of 60 per cent. The room in which the animals were kept was illuminated from 6 a.m. to 6 p.m.

3 Diet. All animals were given the same pellets with a protein content of 18.8 cal. per cent concentrated fish protein (949A Astra-Ewos, Södertälje Sweden). The vitamin content in 10 gram pellets which was the amount consumed by an adult undernourished rat a day was above the daily requirement of an adult normal rat. The vitamin contents in 10 gram pellets in comparison with the normal requirement according to McCoy (1963) within parenthesis were pyridoxin 50 μg (25 μg) niacin 250 μg (0), riboflavin 50 μg (40 μg) and thiamine 50 μg (10 μ) The amount of diet salt constituents in 10 gram pellets was also above the minimum requirement (within parenthesis) of normal adult rats according to McCoy (1963). Thus 10 gram pelets contained 4 mg (0 8 mg) magnesium, 49 μg (40 μg) zink, 2 μg (0.4 μg) cobolt and 25 1 μg (5.3 μg) iodine.

4 Undernutrition. The method of undernutrition was that described by Chow and Lee (1964) This method implies pre- and postnatal undernutrition through a maternal diet restriction amounting to 50 per cent of the amount consumed by a control mother Pregnant rats were given the restricted food amount from the first day of gestation The restriction lasted during pregnancy throughout the suckling period until weaning. After weaning, the nutriment of the young rats was also reduced to 50 per cent of the amount consumed by age-matched control rats up to the age when they were sacrificed. For absolute body weights and bodily growth velocity estimated as the percental increment of normal adult value per one day see figures 1 and 3

No evidence of increased sickliness among undernourished rats was observed in the current experiment.

5 Nutritional rehabilitation. Eight undernourished litters were adjusted to 8 pups per litter at the time of birth and each litter rehabilitated separatly from 0, 5 10 15 20 25 30 and 40 days of age respectively up to 90 days of age Litters were rehabilitated from different ages of close intervals to cover up the time of accelerated fibre growth since this may coincide with a period of increased vulnerability (cf Dobbing, 1968 and Dobbing and Sands, 1971) The rehabilitation consisted of food a libitum to the mother during the suckling period in litters where the rehabilitation was initiated before weaning. After weaning, the 8 litter-mates had free access to food up to 3 months of age when they were investigated.

Ventral and dorsal roots were investigated in rats from all rehabilitated litters, while the sciatic nerve was only investigated from the litter rehabilitated from 0 to 90 days of age For body weights see figure 2.

B Histological procedure

1 Histological technique The animals were anaesthetized with ether They were perfused with a combined paraformaldehyde-glutaraldehyde fixative containing 1 0 per cent paraformaldehyde and 1.25 per cent purified glutaraldehyde in 0 15 M cacodylate buffer of pH 7 15 with added $CaCl_2$ The amount used was 1.5–2.0 ml/gr body weight. This was followed by perfusion with the same volume of a solution four times the above mentioned concentrations. The nerves were then left in situ in the perfused animals for 4 hours, before they were dissected out. They were fixed in concentrated solution for another 12 hours then postfixed in 1 0 per cent OsO_4 for 2 hours, dehydrated and embedded in Epon 812 (cf Karnovsky 1965)

One micron thick sections were cut from a defined level of the sciatic nerve i.e just proximal to the sciatic notch and from the spinal roots in the middle of their lengths, with a LKB-ultrotome and stained with a combination of Mallory azur II and methylen-blue according to Richardson et al (1960)

2 Methodological considerations. A prerequisite of necessity in studies of this kind is to eliminate errors in the histological procedures caused not only by the shrinkage due to fixation and embedding, but also by other artefacts some may be mentioned like beading effects and loss of fibres during histological treatment.

Previous reports on nerve fibre calibre spectra have generally been based on immersion fixed material. It is likely that the immersion fixation will cause errors reflected in the calibre spectrum since fibres centrally located in the nerve will be poorly preserved. (Luse 1960 Condie et al., 1961) This may be partly due to specific permeability properties acting in the perineurial sheaths (Shanthaveerappa, and Bourne 1963) which also might act as osmotic barriers during immersion fixation (Berthold 1968) Furthermore the manipulation with unfixed tissue during dissection preceding the immersion fixation may cause beading effects and fragmented or disrupted myelin sheaths (Ochs, 1963 1965 Hildebrand and

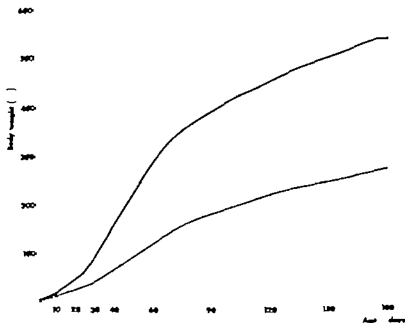


Fig. 1 Absolute body weight gain in control (—) and undernourished (—) rats.

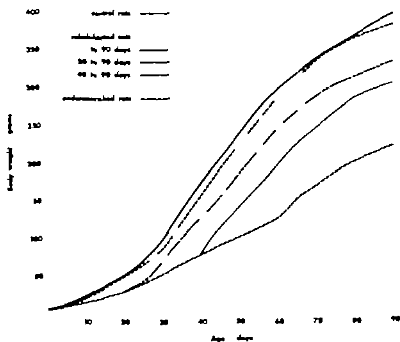


Fig. 2. Absolute body weight gain in rats nutritionally rehabilitated from different ages as compared with control and undernourished rats.

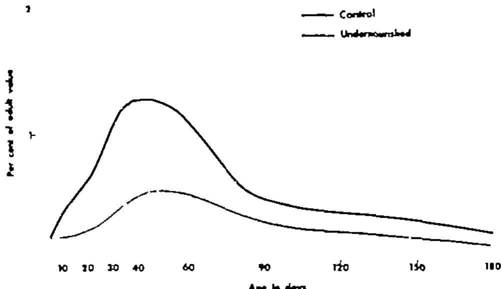


Fig. 3 Body weight increment in control (–) and undernourished ( ) rats measured as percental weight gain per 1 day of normal adult value.

Skoglund 1971). The fixative itself is known to cause various degrees of shrinkage. Rexed (1944) showed in his study using formaldehyde that the concentration of the fixative is of extraordinary importance

The recently usually used fixative in studies of calibre spectrum has been osmium tetroxide. According to Rexed (1944) this fixative causes no shrinkage, however Sherrington (1894) reported a 5 to 7 per cent shrinkage According to Finean (1961) and Sjostrand (1963) using X-ray diffraction techniques, the shrinkage of the myelin period amounts to 10 per cent. Fixation with osmium tetroxide results in a good staining of large fibres whereas small fibres are indistinctly stained and therefore easily overlooked (Hildebrand and Skoglund, 1971 own observations, see figure 5).

Using perfusion-fixation the assumptions for a good preservation are naturally more favourable and mechanically induced artefacts by the dissection procedure can be fully avoided Among perfusion fixatives generally used can be mentioned glutaraldehyde and a combination of paraformaldehyde and glutaraldehyde (Karnovsky 1965) According to Karnovsky the shrinkage caused by this latter combination is unusual despite its high osmolarity

The dehydration and embedding procedure may have caused the greatest artefacts in previous studies on calibre spectrum Rexed (1944) found in his formaldehyde fixed material that the shrinkage due to dehydration and paraffin embedding was 30 per cent Also paraffin embedded osmium tetroxide fixed peripheral nerves displayed a 20 per cent reduction in myelin sheath thickness (Williams and Wendel-Smith, 1960) The author's comparison between unfixed

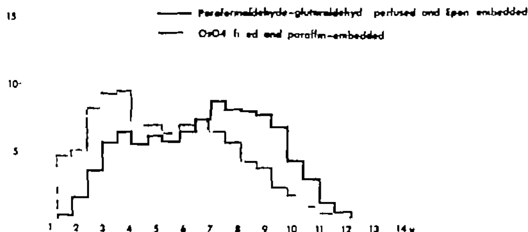


Fig. 4 Calibre spectra illustrating the difference in shrinkage between nerves treated according to the method used in this study (see under Histological technique) and OsO_4-fixed and paraffin embedded nerves.

frozen sections and paraffin sections of osmium fixed sciatic nerve showed a shrinkage in 90-day-old rats among the greatest fibres amounting from 14 to 19 per cent. Osmium fixed material embedded in araldite causes a reduction of the myelin period amounting to 30 per cent according to Finean (1961) and Sjöstrand (1963). A shrinkage of the same magnitude follows after glutaraldehyde fixation and Vestopal embedding (Karlsson 1966).

A drawback of considerable importance occuring in paraffin embedded nerves is the floating apart of nerve fascicles, loss of nerve fibres, and nerve fibres ending up obliquely to the long axis of the nerve, (Skoglund and Romero 1965 own observations see fig. 5) which inevitably will cause a somewhat distorted picture of the fibre distribution. By using perfusion fixation according to Karnovsky (1965) followed by Epon 812 embedding many disadvantages of earlier used methods can be avoided. This method will cause considerably less shrinkage than the earlier used osmium-paraffin method (figure 4).

Own comparisons between perfused and Epon 812 embedded sciatic nerve from one side and cryostat sections of unfixed sciatic nerve from the other side of the same animals showed among the thick fibres, a shrinkage less than 5 per cent in 90-day-old normal and undernourished rats. In this relatively small comparative material the shrinkage in undernourished rats was less than in normally nourished rats. In Epon 812 embedded nerves the loss of fibres were minimal as compared with paraffin embedded material, and fibres ending up obliquely were not observed figure 5).


Fig. 5a.

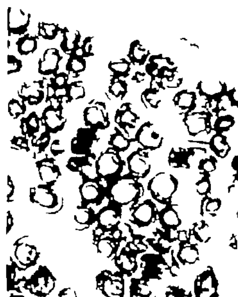

Fig. 5b

Fig. 5 Cross sections of sciatic nerves from 90-day-old control rats treated with different histological techniques, (a) according to the method used in this study (see under Histological technique) (b) OsO_4-fixed and paraffin embedded nerve. Note in (b) fibres ending up obliquely to the long axis and indistinctly stained small fibres.

Consequently this method shows substantially improved assumptions for studies on calibre spectrum of peripheral nerves.

3 Fibre counting technique. Randomly chosen sectors corresponding to approximately one fourth of the cross section of the sciatic nerves and to approximately one half of the cross sections of the ventral and dorsal roots were photographed on Agfa-IFF rollfilm in a Zeiss Photomicroscope The films were enlarged to an overall magnification of 960 times. The entire diameters of the myelinated nerve fibres were measured with a particle size analyzer (Zeiss TGZ 3) according to the method of *Romero* and *Skoglund (1965)* This instrument consists of 48 electromagnetic counters which are connected over a resistance with an adjustable circular light spot. This light spot is projected on a glass screen on which the copies with the enlarged nerve fibres are placed. The light spot is adjusted to the size of the nerve fibre being measured. With a footswitch the current to the corresponding electromagnetic counter is closed and at the same time the current to a sum counter is closed. With this instrument some 500 to 800 fibres can be measured per hour

For slight fibre irregularities measurement calculations were adopted. In a pearshaped fibre the diameter was measured on its base In a sea-star shaped fibre the largest diameter was measured, and if the fibre was oval shaped the minimum diameter was measured (cf Rexed 1944 Romero and Skoglund 1965). Only myelinated fibres were measured.

At the commencement of this study the number of myelinated nerve fibres of the sciatic nerve of age matched control and undernourished rats was compared. Preliminary results showed great individual variations in the number of nerve fibres both in control and undernourished rats. Therefore no conclusions could be drawn from this comparison. Since the number of rats never exceeded three in each group the material basis was considered much too small for an investigation of this kind, and this aspect was not studied further. From other species like the cat, great individual variations are known to occur in fibre number of a defined nerve in animals of the same age (Skoglund and Romero, 1965).

C. Fibre growth velocity

The fibre growth velocity of the total number of myelinated fibres has been estimated on the basis of the calibre spectra. Velocity curves were calculated for the sciatic nerve and the ventral and dorsal roots in control and undernourished rats. Growth velocity was expressed as the percental increment of the normal adult value extrapolated per one day.

D. Statistical methods and presentation of the material

The statistical calculation used for the comparison of the fibre distribution between undernourished and normal rats was test for trend in a contingency table with p-value determination by Edgeworth's expansion and a correction for continuity (Odén and Wedel, 1973). The comparison between the fibre distribution in undernourished sciatic nerves and spinal roots, and normal sciatic nerves and spinal roots was performed so that one single experimental nerve (sciatic or spinal root) was compared to all (2 to 3) nerves of the same type in normal age matched animals.

The presentation of the results is divided into two chapters dealing with the sciatic nerve and the spinal roots respectively. In each chapter the normal development will be described first, followed by the results illustrating nerve fibre development in undernourished and nutritionally rehabilitated rats.

The complete material is presented in tables I–IX, where the number of measured fibres in the different groups are given. To facilitate reading, material of special interest is presented in histograms with references in the text. In these histograms fibres of different diameters have been plotted in percentages of the number of fibres measured against a scale of even microns.

III RESULTS

A. Sciatic nerve

1 Calibre spectrum during postnatal development in control rats. The complete results obtained from the normal development of the sciatic nerve are summarized in table Ia and Ib

As seen from figure 6a, 5-day-old rats showed a fibre distribution which ranged from 0 5 micron to about 4 micron with the fibres concentrated between 1 6 and 2 micron At the age of 10 days the largest fibres had reached a diameter of 5 micron and the peak had shifted to reach 2.2 micron. In 15-day-old rats a bimodal configuration had developed, one peak for thin fibres at 1 8 micron and one for thick fibres at 2.9 micron (figure 7a) After this age a pronounced and continuous increase in thick fibre calibre growth took place up to the age of 30 days. At this age the largest fibres had reached a fibre diameter amounting to 7 5 micron and three peaks had developed, one for thin fibres at 2 2, one for intermediate fibres at 4 1 and one for thick fibres at 5 micron (figure 8a) Between 30 and 40 days the largest fibres increased considerably in diameter and reached at the age of 40 days a diameter of almost 10 micron while the maxima did not change between these ages. From here on a moderate and continuous fibre diameter increase took place up to the age of 90 days, when the largest fibres had reached a diameter of 13 micron and the three peaks had reached 3 9 5 0 and 7.3 micron (figure 9a) Between the ages 90 and 180 days the maxima of the thick fibres as well as of the intermediate fibres continued to show a slow increase in fibre diameters, whereas the maximum of thin fibres remained around 3 9 micron. At the age of 6 months the thick fibres were concentrated around 8 5 micron and the intermediate around 5 6 micron (figure 10a).

2. Calibre spectrum during postnatal development in undernourished rats. A tabulation of the complete results of the fibre growth development in under nourished rats is presented in table IIa and IIb

In the youngest group 5-day-old rats, the fibres of the sciatic nerve were distributed between 0 5 and almost 4 micron with a concentration of fibres around 1 8 micron (figure 6b) At the age of 10 days the fibres were distributed between almost the same diameters as in 5-day-old rats with the maximum still gathered around 1 8 micron In 15-day-old rats, the fibres ranged between 1 and 6 micron with one peak at 2.? micron. Between the ages of 20 and 30 days a continuous and rapid increase in fibre diameters took place and in 30-day-old undernourished rats the largest fibres had reached a diameter of almost 8 micron. Furthermore at this age the calibre distribution showed a bimodal configuration with one maximum for thin

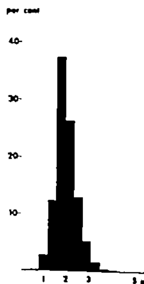
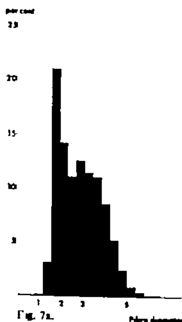




Fig. 6a.   Fig. 6b

Fig. 6. Histograms of sciatic nerves of 5-day-old rats, ( ) control rats, (b) undernourished rats. (For further explanation see text.)



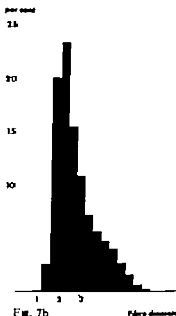
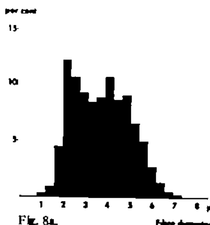


Fig. 7a.   Fig. 7b

Fig. 7 Histograms f sciatic nerves of 15-day-old rats; ( ) control rats, (b) ndernourished rats. (For furthe explanation see text.)



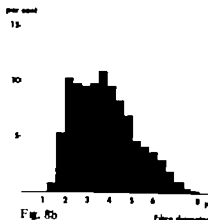


Fig. 8. Histograms of sciatic nerves of 30-day-old rats, (a) control rats, (b) und mourished rats. (For further explanation see text.)

fibres at 2.2 micron and for thick fibres at 3 7 micron (figure 8b) In 40-day-old undernourished rats the maxima were unaltered as compared with 30-day-old rats, whereas the largest fibres had increased to a diameter of 9 5 micron.

Between the ages 40 and 90 days the fibre diameters showed a moderate and uniform increase and the maxima were shifted to reach 3.3 and 6.2 micron respectively in 90-day-old rats. The largest fibres were 11 micron thick at this age (figure 9b). From 90- to 180-day-old experimental rats the fibre diameter increase was slow but still at the age of 6 months the thick fibres showed a tendency to increase in fibre thickness, whereas the thin fibres were still concentrated around 3.3 micron. The thick fibres were gathered around 6 8 micron (figure 10b)



Fig. 9a.

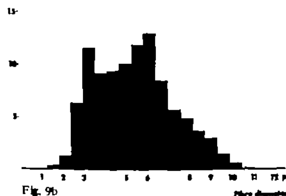


Fig. 9b

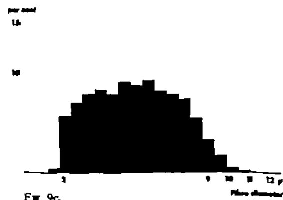


Fig. 9c.

Fig. 9 Histograms of sciatic nerves f 90-day-old rats, (a) control rats, (b) ndernourished rats, (c) rats rehabilitated from 0 to 90 days. (For further explanation see text.)

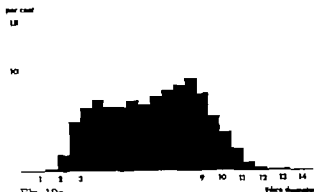


Fig. 10a

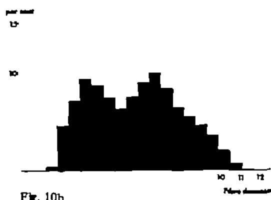


Fig. 10b

Fig. 10 Histograms of sciatic nerves of 180-day-old rats (a) control rats, (b) undernourished rats. (For further explanation see text.)

3 Effect of postnatal nutritional rehabilitation on calibre spectrum. Three rats rehabilitated from birth to 90 days of age showed a calibre spectrum ranging from 1.5 to 11 micron thick fibres. The thick fibres were concentrated around 6 micron and the thin fibres showed a maximum at 3 7 micron. An intermediate peak had reached 5 micron (figure 9c). The complete results of fibre measurements of these rehabilitated rats are presented in table III

4 Comparison of calibre spectrum of control, undernourished, and rehabilitated rats. Comparing the development of sciatic nerve fibre growth of undernourished rats with that of control rats an impaired growth in thickness can be seen of both thin and thick fibres (figure 11a) The very largest fibres were also generally smaller in undernourished rats, however this difference was not that constant when comparing individual rats, as was the difference in fibre diameters of the maxima which occurred very constantly

The effect of early rehabilitation revealed a partial restitution of the calibre spectrum as compared with that of control and undernourished rats. Compare figures 9a, b and c.

The statistical evaluation of the differences between the fibre distribution of the sciatic nerve in undernourished and control animals showed highly significant values in all calculated groups, i.e. 5- 90- and 180-day-old rats ($p < 0.001$) The trend of the significant differences was systematic throughout the calculated groups, indicating a constant difference between the individual rats of the two groups.

Comparing the fibre growth velocity of the sciatic nerve in control and undernourished rats (see figure 11b) a difference in the appearance of the phase of most accelerated growth can be noted. The peak of this phase occurred for the control rats at the age of 10 days, for undernourished rats at the age of 25 days. The growth achievement of undernourished rats represented by the area below the dotted line in figure 11b was decreased as compared with that of control rats.

5. Observations on axonal diameter in control and undernourished rats. In addition to the measurements of the total fibre diameter the axonal diameters of sciatic nerve fibres were compared in two age groups of control and undernourished rats, namely in 30- and 150-day-old rats. The calibre spectrum of axonal diameters of 30-day-old undernourished rats exhibited lesser diameters than that of control rats of the same age Thus in experimental rats 61 per cent of all myelinated axons were less than 2 micron thick, whereas the corresponding figure for control rats was 53 per cent. Even in 150-day-old rats the axonal calibre spectrum of under nourished rats was shifted to lesser diameters as compared with that of control rats (figure 12).

B Spinal roots

1 Calibre spectrum of ventral root (LV) during postnatal development in control rats. The complete results of fibre measurements of the ventral root in control rats are presented in table IVa and IVb

In 5-day-old control rats the fibres of the fifth lumbar ventral root were distributed between 1 and 5 micron with one maximum at about 2 micron, contributed by 32 per cent of all myelinated fibres (figure 13a). At the age of 10 days two maxima had developed, one representing thin fibres at 1 8 micron and one constituted by thick fibres at 3.3 micron. The largest fibres were 5 micron thick (figure 14a) From here on to 20 days of age a very rapid and uniform increase in fibre diameters took place In 20-day-old rats the largest fibres had shifted to reach 8 micron. The two peaks were made up of fibres 2.6 and 6 micron thick. Between 20- and 25-day-old rats the increase in fibre diameter slowed down and showed no increase between 30 and 40 days of age

Between 40- and 150-day-old control rats, the thick fibres showed a moderate increase and reached a maximum of 9 micron at 90 days (figure 15a) At 5 months of age the peak for thick fibres had reached 11 micron. The thin fibres increased slowly from 40 up to 60 days and constituted at this age a maximum at 3.3 micron.

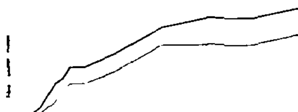


Fig. 11a.



Fig. 11b

Fig. 11a. Increase in size of fibres comprising the peak fo thick fibres of the sciatic nerve of control (–) and undernourished (--) rats.

Fig. 11b Growth velocity curves of fibre thickness growth of total number of myelinated fibres in sciatic nerves of control (–) and undernourished ( ) rats (For further explanation see text.)

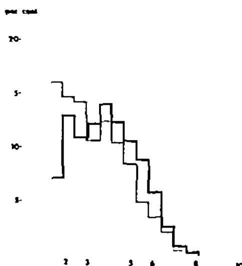


Fig. 12 Histogram illustrating the axonal diameters of sciatic nerve in control (–) and undernourished (--) rats 150 days old

After the age of 2 months the thin fibres did not show any increase until the sixth month, when these had shifted to reach a maximum around 3 9 micron. The largest fibres had reached 16 micron and the maximum of thick fibres consisted of fibres 12 micron thick (figure 17a)

2 *Calibre spectrum of dorsal root (LV) during postnatal development in control rats.* A tabulation of the complete fibre measurements in dorsal roots of control rats is presented in table Va and Vb

Five-day-old rats showed a fibre distribution between 0.5 and 4 micron with one peak at 2.2 micron (figure 13b) In 10-day-old rats the nerve fibres of the dorsal root were distributed in the same way (figure 14b) Between the ages 10 and 15 days the fibre diameters increased rapidly The largest fibres had almost reached 8 micron in 20-day-old control rats. At this age the calibre spectrum showed a bimodal configuration with one peak for thin fibres around 2 micron and one for thick fibres at 4 1 micron. After 20 days of age the increase in fibre diameter declined and between 30 and 40 days no increase of thick fibres took place except for the very largest fibres which had shifted to 9 micron. At 40 days a third intermediate peak had developed at 3 micron From this age up to 180-day-old rats a slow and continuous increase could be noted Hence at the age of 90 days the fibres were distributed between 1.5 and 12 micron with the peaks at 2.7 5.5 and 7.3 micron (figure 16a) Still at the age of 6 months both thin and thick nerve fibres showed a tendency of increase In the oldest rats investigated the maxima had reached 3.3 6.7 and 8 4 micron. The largest fibres had shifted to reach 14.5 micron (figure 17c).

3 *Calibre spectrum of ventral root (LV) in undernourished rats during postnatal development.* A tabulation of the complete results of the fibre measurements of the ventral roots in undernourished rats is presented in table VIa and VIb

In the youngest group of undernourished rats investigated 5 days of age the fibres of the ventral root ranged from 0.5 to 4 micron with one peak at 1 8 micron formed by 44 per cent of all fibres (figure 13c). In 10-day-old rats the fibres were distributed between the same diameters but showed two peaks, one at 1.8 and one at 2.6 micron (figure 14c). In undernourished rats 15 days of age the ventral root fibres were distributed between 1 and 6.5 micron. The maximum for thin fibres was still gained by fibres 1 8 micron thick, while the maximum for thick fibres had shifted to 3.3 micron Between the ages 15 and 25 days the thick fibres showed a rapid and uniform increase and had reached a peak at 6 micron at the end of this period The peak for thin fibres was contributed by fibres 1.9 micron thick Between 25 and 40 days of age no increase in growth of thick fibres took place However at 40 days the thin fibres had shifted to reach a peak at 2.2 micron From 40 days onwards the thick fibres showed a relatively slow but continuous increase in fibre growth. This was also true for the thin fibres Accordingly at the age of 90 days the maxima had reached 3.3 and 7.9 micron respectively (figure 15b) In 180-day-old undernourished rats the peaks were located at 3 9 and 11 4 micron for thin and thick fibres respectively The largest fibres had now reached 14 5 [illegible] (figure 17b)



Fig. 13a.



Fig. 13b

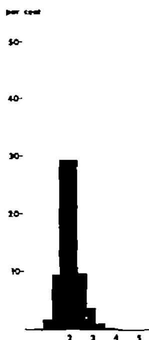
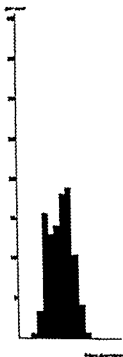


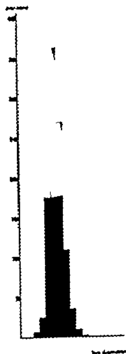
Fig. 13c.

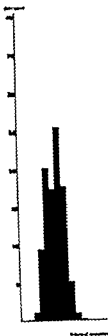


Fig. 13d

Fig. 13 Histograms of ventral and dorsal roots (LV) of control and undernourished rats 5 days of age (a) ventral root of control rats, (b) dorsal root of control rats, (c) ventral root of undernourished rats, (d) do sal root of undernourished rats. (Fo further explanation see text)


Fig. 14a


Fig. 14b.


Fig. 14c.

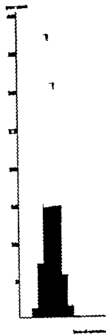

Fig. 14d

Fig. 14 Histograms of ventral and d rsal roots (LV) of control and undernourished rat 10 days of age ( ) ventral root of control ats, (b) dorsal root of control rats, ( ) ventral root of undernourished rats, (d) dorsal root f undernourished rats (For further explanation see text.)



Fig. 15a

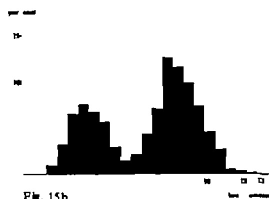


Fig. 15b

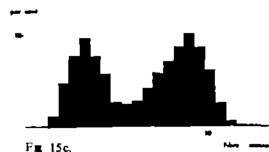


Fig. 15c.

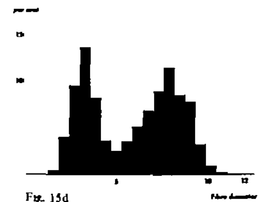


Fig. 15d

Fig. 15 Fibre distribution of ventral roots (*LV*) in 90-day-old rats, (a) *control rats*, (b) undernourished rats, (c) rat nutritionally rehabilitated from 5 to 90 days, (d) rats nutritionally rehabilitated from 40 to 90 days. (For further explanation see text.)

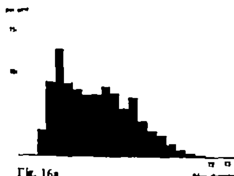


Fig. 16a.

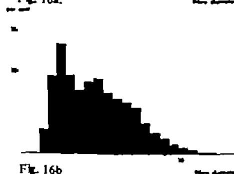


Fig. 16b

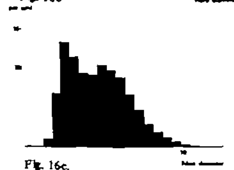


Fig. 16c.

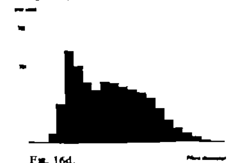


Fig. 16d.

Fig 16 Fibre distribution of dorsal roots (LV) in 90-day-old rats, ( ) control rats, (b) under nourished rats, ( ) rats nutritionally rehabilitated from 5 t 90 days, (d) rats nutritionally rehabilitated from 40 t 90 days. (For further xplanation see text.)

4 Calibre spectrum of dorsal root (LV) in undernourished rats during postnatal development. The complete material is presented in table VIIa and VIIb

In 5-day-old rats the fibres of the dorsal root were spread between 0.5 and 3.5 micron with one maximum at 1 8 micron (figure 13d) In 10-day-old rats the largest fibres had reached a total diameter of 4 micron but still only one maximum was visible at 1 8 micron (figure 14d) At the age of 20 days the maximum had first shifted to 2.2 micron and at this age the largest fibres were 6.5 micron thick. At the age of 25 days two peaks appeared for the first time, one for thin fibres at 1 8 micron and one for thick fibres at 4 micron. Between 20 and 25 days the thick fibres showed a rapid increase At the end of the first postnatal month the thick fibres had shifted to a maximum at 4 4 micron Between 30 and 40 days the thick fibres of the dorsal root showed no increase (figure 19a) whereas the thin fibres showed a rapid increase in fibre diameter and reached at the end of this period 2.2 micron. From 40 days of age the undernourished rats showed a slow and relatively uniform increase in diameter of thick fibres. The thin fibres showed no increase between 40 and 60 days, and after this period the increase was slow At the age of 90 days the peaks were located at 2.7 and 5 micron (figure 16b) At the end of the observation period, i.e. in 180-day old undernourished rats, the maxima had reached 3.3 and 6 1 micron. The largest fibres had reached a diameter of 12 micron (figure 17d).

5 Comparison of calibre spectrum of normal and undernourished rats.

Ventral roots. Comparing the fibre growth development of undernourished rats with that of normally nourished control rats, a deficit in fibre growth can be noted among thick fibres, whereas the deficit in fibre growth of thin fibres is small The timing of the most rapid growth of the nerve fibres occurred at the age of 20 days, both in control and undernourished rats (figure 18b). However the growth-spurt in the undernourished rats occured to a lesser extent (figure 18b) Statistical treatment of three age groups 5- 90- and 180-day-old rats showed highly significant differences ($p < 0\ 001$) between individual undernourished and control rats. The trend of the differences was systematic in the groups mentioned, indicating a constant difference between the individual rats of the two groups.

Dorsal roots. Comparing the fibre growth development of the dorsal roots of undernourished rats on one hand and control rats on the other great differences are obvious between the thick fibres whereas the thin fibres show no differences.

At the age of 180 days the thick fibres of undernourished rats have reached only some 70 per cent of the normal diameter (figure 19a) In the dorsal root the timing of the phase of accelerated growth was retarded by 15 days in undernourished rats, and occurred to a markedly lesser extent (figure 19b).

Statistical evaluation of the same age groups as those evaluated for the ventral root also showed, among the dorsal roots, highly significant differences ($p < 0\ 001$) between undernourished and control rats concerning the fibre distribution The trend of the differences here was also systematic indicating a uniform difference between individual rats throughout the groups evaluated.

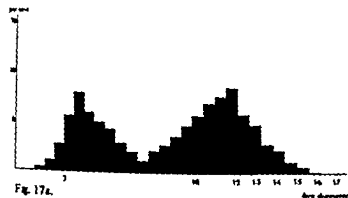


Fig. 17a.

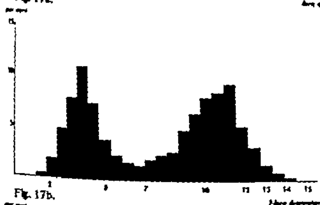


Fig. 17b.

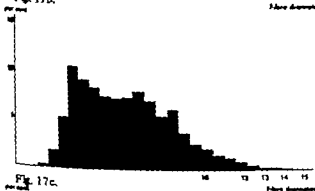


Fig. 17c.

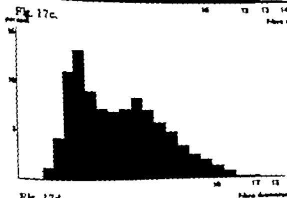


Fig. 17d.

Fig. 17 Fibre distribution of ventral and dorsal oot (LV) in 180-d y-old rats, ( ) ventral root of control rats, (b) ventral root of un-dernourished rats, ( ) dorsal root f control ats, (d) dorsal root of ndernourished rats. (For further explanation see text.)



Fig. 18a



Fig. 18b

Fig. 18. Diagram (a) shows the increase in size of fibres comprising the peak for thick fibres of the ventral roots (LV) of control (–) and undernourished ( ) rats. Diagram (b) shows the growth velocity curves of fibre thickness growth of the total number of myelinated fibres in ventral roots of control (–) and undernourished ( ) rats. (For further explanation see text.)



Fig 19a

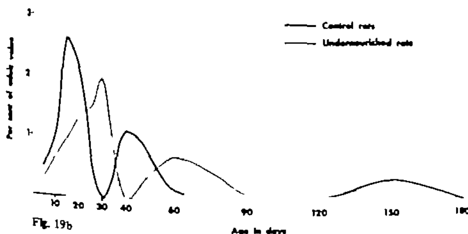


Fig. 19b

Fig. 19 Diagram ( ) shows the increase in size f fibres comprising th peak for thick fibres of the dorsal roots (LV) of control (–) and ndernourished (---) rats. Diagram (b) shows the growth elocity of fibre thickness growth of the total number f myelinated fibres in dorsal roots of control (–) and ndernourished (---) rats. (For further explanation see text.)

6 Effect of postnatal nutritional rehabilitation on calibre spectrum of ventral root (LV). The complete results are given in table VIII

In all undernourished rats in which rehabilitation was initiated between 0 and 15 days of age (early rehabilitation) the calibre spectra of the ventral roots showed almost the same distribution of the fibres at the age of 90 days. Only minimal differences can be seen from the histograms of these rats. The fibres were distributed between 1 and 12–13 micron with two distinct peaks, one for thin fibres at 3.3 micron and one for thick fibres at 9 micron (figure 15c). In undernourished rats rehabilitated from 25 30 and 40 days of age up to 90 days (late rehabilitation) the calibre spectra ranged between 1 and 11–13 micron The maxima for thin fibres were located at the same diameter as the corresponding maxima of undernourished rats subjected to early rehabilitation (i.e. from 0 5 10 and 15 days of age) whereas the maxima for thick fibres were located at 7,9 micron (figure 15d) In rats rehabilitated from 20 days to 90 days of age the maximum for thin fibres was located at 3.3 micron and the maximum for thick fibres at 8 4 micron.

7 Effect of postnatal nutritional rehabilitation on calibre spectrum of dorsal root (LV). The complete material is presented in table IX.

The calibre spectra of the dorsal roots of all undernourished rats subjected to rehabilitation were distributed between 1 and 10–11,5 micron All animals of the entire groups of rehabilitated rats showed two maxima which were gathered around 2.7 micron for thin fibres and around 5 micron for thick fibres. Rats in which rehabilitation was initiated between 0 and 20 days of age (early rehabilitation), showed calibre spectra with a greater number of thin and intermediate sized fibres than the calibre spectra of rats rehabilitated from later ages (figure 16c) Conversely rats rehabilitated from 25 30 and 40 days of age up to 90 days of age (late rehabilitation) showed a greater number of thick fibres in their calibre spectra of the dorsal root than rats subjected to early rehabilitation (figure 16d)

8 Comments. Ventral roots. The calibre spectra of the ventral roots in rats subjected to early rehabilitation examined at the age of 90 days showed a fibre distribution similar to that of the ventral root in control rats of the same age (compare figures 15a and 15c) whereas rats subjected to late rehabilitation, examined at 90 days of age showed calibre spectra similar to that of the ventral root in equally old undernourished rats.

Using statistical comparisons between the fibre distribution of the ventral roots in rats rehabilitated between 5 and 90 days of age and that of control rats 90 days old, it was found out of 9 possible comparisons between individual rats of the two groups, 5 showed significant differences and 4 did not. However the trends of the significant as well as the nonsignificant differences were not systematic. This indicates that no differences in the fibre distributions between these two groups could be obtained. The opposite relationship was valid for the comparative conditions between the fibre distributions in the ventral roots of rehabilitated rats and undernourished rats, where the trend was systematic throughout these two groups. In this case with regard to fibre distribution the trend indicates that the

two groups were different and separate from each other (see table X) In addition it can be mentioned that statistical comparisons between the fibre distribution of individual rats belonging to the same control groups showed significant differences in some cases and not in some reflecting individual differences.

Dorsal roots. Comparing the fibre distribution of the dorsal roots of rats rehabilitated from different ages up to 90 days of age with control and undernourished rats of the same age the maxima were located at the same diameters as those of undernourished rats. In rats subjected to rehabilitation like in undernourished rats, at third maximum never developed Rats subjected to early rehabilitation up to the age of 90 days, showed a slight increase in number of thin and intermediate sized fibres, whereas in rats subjected to late rehabilitation the free access to food was profited mainly by the thick fibres which increased in number when compared with those of undernourished rats.

Statistical comparisons between rats being rehabilitated from 5 to 90 days of age and undernourished rats 90 days old showed significant differences in their fibre distributions in 6 out of 9 cases. The trend between these two groups was not systematic which indicates a similarity between the two groups. The converse relationship was true with regard to the comparison between the fibre distribution in rats of the same rehabilitated group and that of control rats 90 days old. In this latter case the trend was systematic throughout all 9 possible comparisons. This indicates that these two groups are different as far as their fibre distribution of the dorsal root is concerned. For comparison see table X.

IV GENERAL DISCUSSION

The general pattern of the normal development of the calibre spectra of the sciatic nerve and its roots in the rat corresponds to that know from other species. Thus, the results obtained from the rat may be compared with previous results on cat (Skoglund and Romero 1965) and on man (Rexed, 1944) provided that corresponding developmental stages and not postnatal ages of the different species are considered.

The ventral root of the rat established a bimodal adult pattern of its calibre spectrum as early as at the age of 10 days with one peak for thin fibres and one peak for thick fibres. In the cat it has been shown that the thin fibres represent gamma-fibres (Eccles and Sherrington, 1930) innervating intrafusal muscle fibres in muscle spindles (Leksell 1945) and the thick fibres alfa-fibres innervating the main mass of extrafusal muscle. Compared to the ventral root, the dorsal root exhibited a slower development. The adult pattern of fibre distribution was reached at 40 days of age when three maxima were discerned, corresponding to I II and III fibres according to the classification proposed by Lloyd (1943) Also the development of the calibre spectrum of the sciatic nerve was slow compared to the ventral root. At the proximal unbranched level investigated in this study a bimodal configuration with two distinct peaks was reached at the age of 15 days, while the adult pattern with three maxima was not established before the age of 30 days. The fibre types included in a mixed peripheral nerve such as the sciatic nerve are of *course of heterogenous origin and therefore no conclusions could be drawn* concerning the relationship of the different maxima to defined fibre types.

Summarizing the development of the adult pattern of fibre distribution of the three nerves investigated it can be stated that the rat, like the cat (Skoglund and Romero 1965) showed the fastest development of the ventral root whereas both the dorsal root and the sciatic nerve showed a considerably slower development.

In man (Rexed, 1944) *like in* cat (Skoglund and Romero 1965) *the increase in* fibre size takes place during a relatively long span of life In man the dorsal root of *the first sacral segment still shows a tendency to increase in fibre thickness beyond* the age of 25 years. As for the cat, even young adult animals still show an increasing fibre size At the age of 180 days corresponding to one fourth of the average life expectance of the rat, the thick fibres were thicker than in 150-day-old rats. It is thus obvious that the rat like the cat and the man displays an increase in fibre thickness proceeding into adult life.

A comparison of the normal development of the sciatic nerve in rat with that in pre and postnatally undernourished rats revealed marked differences. In the sciatic

nerve of undernourished rats the calibre growth of all fibres seemed to be retared but the retardation was more marked in the thick than in the thin fibres. Nerve fibres of intermediate size did not develop a well defined maximum in undernourished rats. When comparing the number of myelinated fibres in control and undernourished rats of the same ages, no significant difference was demonstrable. However no definite conclusions from such a comparison can be drawn since the number of rats examined in each age group was restricted to 3 animals, which was considered to be too small. It is known that considerable differences occur in fibre number between individual animals of the same age as shown by Skoglund and Romero (1965) for the cat and indicated for the rat by the author's observations.

The functional heterogenity of the fibres forming the sciatic nerve and the differences noticed in the effect of undernutrition on thick and thin fibres demanded an investigation of the corresponding motor and sensory roots.

The postnatal development of spinal roots in undernourished rats was markedly retarded. This retardation was more pronounced in the dorsal than in the ventral root. In the dorsal root of 180-day-old undernourished rats the thick fibres showed a 30 per cent reduction in fibre thickness as compared with those of control rats. The intermediate sized nerve fibres did not develop a defined maximum in the dorsal root of undernourished rats. The location of the peak for thin fibres of the dorsal root was not affected by undernutrition.

In the ventral root, the thick fibres were markedly reduced in growth of thickness, whereas the thin fibres showed only a slight reduction in their calibre growth.

It is tempting to assume that the physiological consequence of the deficits found in nerve fibre calibres of peripheral nerves in undernourished rats will be manifested as reduced conduction velocities, since it is known that there exists a relationship between nerve fibre calibre and conduction velocity (Erlanger and Gasser 1937, Hursh, 1939). In order to explore this possibility examinations are now in progress concerning the conduction velocity of peripheral nerves in undernourished rats (Jankowska and Sima)

In rats undernourished early in life, a reduced number of myelin lamellae of the sciatic nerve fibres has been ultrastructurally demonstrated by Clos and Legrand (1970) and by Hedley Whyte and Meuser (1971). Clos and Legrand found that for axons of about the same circumference the number of myelin lamellae was markedly reduced in undernourished rats. This observation was interpreted to indicate a direct effect of undernutrition on the process of myelination. However the results obtained by Hedley Whyte and Meuser showed that not only the myelination but also the circumferential growth of the axon is affected by early undernutrition. This is in agreement with the author's observations on the axonal diameter which appeared to be decreased in undernourished rats compared with age-matched control rats. The latter observations correspond well with the findings by Haltia (1970) which showed a reduced protein and RNA content of the motor neurons of the anterior horn in undernourished rats, indicating an impaired protein

metabolism. It may be assumed that an impaired protein metabolism will not only affect the growth of the perikaryon but also the growth of its axon Furthermore the observed deficit in axonal growth in undernourished rats may in turn be responsible for an impaired myelination since it is known that the process of myelination is dependent on axonal protein flow and axonal growth (Friede 1972).

In order to find out whether the observed deficits in nerve fibre development in undernourished rats were of a reversible nature or not undernourished rats were nutritionally rehabilitated from birth and from early postnatal ages.

Sciatic nerves of rats rehabilitated from birth to 90 days of age showed a partial restoration of the calibre spectrum when compared with age-matched control rats. This partial restoration of the calibre spectrum of the sciatic nerve is in accordance with the ultrastructural findings of Hedley Whyte (1973) which showed that the reduced axonal circumference and number of myelin lamellae in sciatic nerves of undernourished rats were only partially recovered by subsequent rehabilitation

Ventral roots of previously undernourished rats rehabilitated from various ages between birth and 40 days up to adult age showed when rehabilitation was initiated before the period of accelerated fibre growth (around the age of 20 days) a complete restoration of the calibre spectrum Contrary to this, when rehabilitation was initiated after the period of accelerated fibre growth no restoration of the calibre spectrum was observed however the calibre spectrum was similar to that of age matched undernourished rats. These findings are in agreement with the hypothesis launched by Dobbing (1968) concerning the brain growth, that the vulnerable period coincides with the time of accelerated growth.

In contrast to the complete recovery of the ventral root after early rehabiliation, nutritional rehabilitation even if initiated at birth, showed only minimal effects on the calibre spectrum of the dorsal root. When rehabilitation was initiated before 20 days of age i.e just before weaning thin fibres increased slightly in number When rehabilitation was initiated after this age a slight increase in number of thick fibres was observed whereas thin fibres did not show any increase in number when compared with age-matched undernourished rats. The rehabilitation did not change the location of the maxima characteristic for undernourished rats and did not result in statistically significant changes in fibre distribution compared with the calibre spectrum of undernourished rats. It is thus obvious that undernutrition causes an early inititated irreversible damage to the dorsal root.

Looking at the growth velocity curves of the sciatic nerve and its roots of both normal and undernourished rats (figures 11 18b and 19b) it can be seen that the development of nerve fibre calibres show discontinuous courses. This discontinuous increase in nerve fibre thickness may reflect changes in the synthesis of proteins essential for the growth of neurons. This hypothesis is supported by observations stating that the rate of increase of substances necessary for protein synthesis, e.g. neuronal RNA (Haltia, 1970) and those included in the free amino acid pool (Miller 1969) also show discontinuous courses during development.

Comparing the occurrence of the phase of the most accelerated growth in undernourished and control rats principal differences can be noticed between the

ventral root on one hand and the dorsal root and the sciatic nerve on the other hand. In the ventral root the phase of fastest growth in undernourished rats coincides chronologically with that in control rats. In the dorsal root and the sciatic nerve this phase was delayed as compared with that of respective nerves of control rats. In all three nerves of undernourished animals the ultimate growth achievement represented by the area enclosed between the growth velocity curve and the time axis was reduces. This is obvious when comparing the areas below the curves representing the nerves of undernourished rats with the areas below the curves representing the nerves of control rats.

According to Dobbing and Sands (1971) biosynthetic mechanisms involved in growth phenomena may be present only at the time of growth spurt. When this phase is passed achieved deficits in growth will persist. In the present study we have seen that undernutrition caused persistent deficits in the dorsal root and partially persistent deficits in the sciatic nerve whereas the calibre spectrum of the ventral root could easily be restored by early initiated rehabilitation. The irreversible effects on the dorsal root and the partially irreversible ones on the sciatic nerve could however not be prevented even if rehabilitation was initiated long before the phase of accelerated growth. The partial restitution of the nerve fibre calibres of the sciatic nerve of rehabilitated rats is presumably due to the summation of the in principle different effect of rehabilitation observed between the fibres of the ventral and the dorsal spinal roots, since they both merge into the mixed sciatic nerve.

The delay in the phase of fastest growth in the dorsal root and the sciatic nerve may be responsible for the retarded appearance of defined maxima of large fibres in undernourished rats.

The reason for the difference in occurrence of the rapid growth phase noticed between the ventral and dorsal roots of undernourished rats in relation to the occurrence of respective phases in control rats can only be a matter of speculation. It is obvious that diet provides the necessary precursor pools, like the amino acid pool, for macromolecular synthesis and that a reduction in pool size may affect the synthetic rate. This may be affected differently in anterior horn motor neurons and spinal ganglion cells, since possible disturbances in enzym pattern, in energy metabolism or in hormonal regulation may interfere differently with amino acid incorporation and macromolecular synthesis in these cells (cf. Miller 1969) and therefore be responsible for the different effect of undernutrition upon fibre growth in ventral and dorsal roots.

V SUMMARY

The effect of experimental pre- and postnatal undernutrition on the development of nerve fibre calibres of the sciatic nerve the ventral and dorsal roots of the fifth lumbar segment of the rat was systematically investigated. The main findings were as follows.

1 Undernutrition initiated early in life causes deficits in the nerve fibre calibre growth in the sciatic nerve and its roots In all nerves investigated the thick fibres were more affected in calibre growth by undernutrition than the thin fibres.
2. The impairment in nerve fibre calibre growth of the dorsal root proved to be irreversible after early initiated nutritional rehabilitation In the sciatic nerve the fibre growth was partially restored and in the ventral root it was completely restored after nutritional rehabilitation
3 The peaks of accelerated growth of the dorsal root and the sciatic nerve of undernourished rats were delayed as compared with those of control rats. In the ventral root of undernourished rats the peak of accelerated growth occurred at the same age as in control rats.

Thus experimental undernutrition induces reversible deficits in the calibre growth of the ventral root fibres whereas in the dorsal root the fibre calibre growth is permanently distorted by undernutrition. In the sciatic nerve the impairment of calibre growth caused by undernutrition is partially reversible

Acknowledgements I wish to express my sincere gratitude to Ass. Prof Patrick Sourander who initiated this study and for most valuable aid and criticism during the investigation. I also wish to express my sincere thanks to Miss Kristina Müntzing and Miss Margareta Persson for their skilful histotechnical assistance and to Fil. lic. Anders Odén for efficient statistical work. To Prof. Tore Arwill my sincere thanks for kindly lending me the particle size analyzer used in the present study The work was supported by a grant from the Swedish Medical Research Council projects No. B72 12X 3488-01A and B73 12X-3488-02B and by a grant from the Medical Faculty University of Goteborg.

VI REFERENCES

Berthold, C.H. A study on the fixation of large mature feline myelinated ventral lumbar spinal-root fibres. Acta Soc. Med Uppsal 73 Suppl 9 1 36 1968

Chow B.F and Lee C.J Effect of dietary restriction of pregnant rats on body weight gain of the offspring. J Nutr 8 10 18 1964

Clos, J Legrand J Influence de la déficience thyroidienne et de la sous-alimentation sur la croissance et la myelinisation des fibres nerveuses du nerf sciatique chez le jeune rat blanc Brain Res 22 85–297 1970

Condie, R.M Howell, A F and Good R.A Studies on the problem of preservation of myelin sheath ultrastructure evaluation of fixation dehydration and embedding techniques J Biophys. Biochem. Cytol. 9 4 9 443 1961

Dobbing, J The influence of early malnutrition on the development and myelination of the brain. Proc. Roy Soc Biol 159 503 509 1964

Dobbing, J Vulnerable periods in developing brain In Applied Neuro [illegible] Eds A.N Davison and J Dobbing. Blackwell Oxford and Edinburgh [illegible] 287 316 1968.

Dobbing, J and Sands, J Vulnerability of developing brain IX The effect of nutritional growth retardation on th timing of brain growth spurt [illegible] Neonat 19 363 378 1971

Dobbing, J and Smart, J.L Early undernutrition, brain development and behaviour Clinics in Developmental Medicine No 47 Ethology and Development, Edited by S.A Barnett Heinemann London 1973

Eccles J.C and Sherrington, C.S. Number and contraction values of individual motor units examined in some muscles of th limb Proc Roy Soc Biol 106 326–357 1930

Erlanger J and Gasser H S Electrical Signs of Nervous Activity Univ Penn Press Philadelphia 1937

Finean, J.B The nature and stability of nerve myelin [illegible] Cytol 12 303–334 1961

Friede R.L Control of myelin formation by axon caliber (with a model of the control mechanism) J Comp Neur 144 233–252 1972

Hallin M Postnatal development of spinal [illegible] in normal and undernourished rats. A quantitative cytochemical study Acta Physiol Scand Suppl. 352, 1970

Hallin, M and Sourander P The effect of early undernutrition on neuronal maturation. J Neuropath. Exp Neur 29 144, 1970 a

Hallin, M. and Sourander P The effect of undernutrition [illegible] nucleic acid content of growing nerve cell. Proc. VIth Intern [illegible] Neuropath Masson et Cie Paris p 47 1970 b

Hedley Whyte E T Myelination of rat sciatic nerve [illegible] and cholesterol biosynthesis inhibition [illegible] 1973

Hedley Whyte E T and Meuser C.S The effect [illegible] of rat sciatic nerve Lab Invest., 24 156 161 1971

Hildebrand C. and Skoglund S Calibre spectra of some fibre tracts in the feline *central nervous system during postnatal development.* Acta Physiol Scand. Suppl. 364 5–43 1971

Hursh J B Conduction velocity and diameter of nerve fibres. Am J Physiol. 127 131–139 1939

Karlsson, U Comparison of the myelin period of peripheral and cental origin by electron microscopy J Ultrastruct Res. 15 451–468 1966

Karnovsky M J A formaldehyde-glutaraldehyde fixative of high osmolarity for use in electron microscopy J Cell Biol. 27 137A 1965

Leksell L The action potential and exitatory effect of the small ventral fibres to skeletal muscle. Acta physiol Scand 10 Suppl 31 1945

Lloyd D P C Neuron patterns controlling transmission of ipsilateral hind limb reflexes in cat J Neurophysiol. 6 293–315 1943

Luse S A. *Fixation and embedding of mammalian brain and spinal cord for* electron microscopy J Ultrastruct. Res. 4 108–112 1960

McCance R A and Widdowson E M (Eds) Calorie deficiencies and protein deficiencies J & A Churchill London 1968

McCoy R.H Dietary requirements of the rat In The rat in laboratory investigation. Eds. E.J Farris and J Q Griffith Hafner New York Pp 68–103 1963

Miller S.A Protein metabolism during growth and development In Mammalian Protein metabolism Vol III Ed H.N Munro Academic Press New York and London 1969

Ochs S Beading phenomena of mammalian myelinated nerve fibres. Science 1 599–600 1963

Ochs, S Beading of myelinated nerve fibres. Exp Neurol. 12 84–95 1965

Odén, A and Wedel, H Arguments for Fisher's permutation test Dept. of Chalmers institute of Technology Göteborg. No 1 14 1973

Platt B S and Stewart R C S Effects of prote deficiency on Morphological changes in the nervous system d Child 174–192 1969

Rexed B Contributions to the knowledge of developmen peripheral nervous system in man A study of scope of sy investigations into the fibre size in perip psychiat Suppl. 33 Stockholm 1944

Richardson K.C Jarett, L. and Finke E.H P y ultrathin sectioning in electron microscopy Stain 3–32

Romero C and Skoglund S Methodological stud In *nerve fibre diameters. Acta Morph Neerl. Scand.*

Shanthaveerappa, T R., and Bourne G.H The perL N significance. Nature (Lond.) 199 577–579 1963

Sherrington C S On the anatomical constitution of remarks on recurrent fibres in the ventral spinal 17 211–258 1894

Sjöstrand F S The structure and formation of the myel Demyelination I Eds. A S Rose and C M Pearson 1–43 1963

Skoglund S and Romero C Postnatal growth of sp morphological study in the cat with physiological Scand Suppl 260 1965

Williams, P L. and Wendell-Smith, C P The use of fixed quantitative studies of peripheral nerve Quart. J Micr

Winick M Changes in nucleic acid and protein content growth Pediat Res 2 352–355 1968

Winick, M. Malnutrition and brain development. J. Pediatrics 74: 667–679, 1969.
Winick, M. and Noble, A. Quantitative changes in DNA, RNA and protein during prenatal and postnatal growth in the rat. Develop. Biol. 12: 451–466, 1965.
Winick, M. and Rosso, P. The effect of severe early malnutrition on cellular growth of human brain. Pediat. Res., 3: 181–184, 1969.

VII TABLES

Table 1a. I ibre distribution in sciatic nerves of n rmal rat 5–40 days old.

Age in days	Animal nr	0,42–0,80	0,80–1 19	1 19–1,57	1,57–1 96	1 96–2,33	2,33–2,72	2,72–3 10	3 10–3,49	3 49–3 87	3,87–4,25	4,25–4,63	4,63 5,02
5	244/1	4	48	173	644	474	2..9	110	38	6	3		
	244/2	4	42	228	561	389	190	62	17	4			
10	289/2	0	0	17	279	373	204	143	98	80	54	12	6
	289/5	0	0	19	233	416	251	236	151	86	61	22	6
	289/7	0	0	44	485	490	284	185	96	50	7	2	
15	222/3	0	2	68	428	360	287	290	262	253	182	92	34
	222/4	0	0	18	202	274	213	280	273	269	194	130	62
	222/5	0	1	115	521	282	218	245	208	192	179	114	64
20	221/4	0	4	17	72	204	215	240	298	305	252	176	136
	221/5	0	8	40	87	211	282	287	236	329	261	264	223
	221/6	0	1	29	140	302	402	423	433	362	253	178	114
25	223/5	0	6	33	129	224	166	191	257	264	276	234	205
	223/8	0	0	26	109	212	139	144	139	190	191	174	179
30	213/5	2	6	11	77	278	264	238	221	211	229	183	185
	213/6	1	12	31	133	225	183	149	146	168	242	201	218
	213/7	0	1	18	103	353	305	268	232	248	277	230	239
40	222/2	0	0	6	77	127	121	122	126	126	133	141	175
	??/6	0	0	21	111	99	85	86	134	120	163	168	236
	/7	0	0	17	91	95	98	113	164	127	120	148	129

I ibre dist ibution in sciatic nerves of normal r t 60–180 days old

e in day	Animal nr	1 26 1 83	1 83–2,42	2,42–2,98	2,98–3,56	3 56–4 14	4 14 4 70	4 70–5 28	5,28–5 86	5 86–6 43	6 43–7 01	7,01–7,58	7,58 8 16
60	221/3	8	194	274	274	231	216	185	221	235	245	162	87
	221/7	?6	201	241	230	217	278	?93	324	263	167	99	52
	221/8	4	169	17	249	27	250	242	250	226	216	137	74
90	200/1	11	?8	113	146	162	136	180	151	171	172	218	229
	200/2	11	44	127	191	237	200	196	214	234	257	315	256
	200/3	14	42	78	162	167	154	168	139	157	214	219	221
120	139/2	9	94	121	123	139	133	115	137	122	137	147	158
	139/6	15	101	174	2	251	271	210	209	225	250	251	281
	215/5	0	6	22	57	121	146	137	145	129	111	121	136
150	243/1	4	85	115	117	174	163	164	168	148	145	186	213
	243/2	4	115	161	142	230	175	171	179	05	179	281	297
	243/3	1	56	87	1 4	148	147	161	154	148	148	147	152
180	245A/2	3	25	100	115	144	134	125	135	119	154	175	190
	245A/6	3	27	92	134	135	109	115	125	116	140	153	176
	245A/7	10	64	170	221	245	229	232	258	256	254	265	268

[illegible]–5,40	5,40–5,79	5,79–6,16	6,16–6,55	6,55–6,93	6,93–7,32	7,32–7,70	7,70–8,07	8,07–8,46	8,46–8,85	8,85–9,23	9,23–9,62	Number of fibres measured
												1729
												1497
1												1267
1												1482
												1643
13	3											2274
73	6	5	1									1950
21	14	2										179
54	24	12	1	2								2054
185	105	36	10	1	2							2567
38	16	6										2709
141	109	57	13	6	1							312
145	112	77	36	19	1	4						1897
153	124	64	32	8	2							2288
146	117	65	36	70	5							2098
164	96	62	23	10	4	2						2635
103	144	166	149	130	94	91	44	30	18	8	7	2240
205	108	157	116	117	87	51	24	11	6	4	1	2190
130	136	152	139	97	79	53	26	13	5	3		1943

8,16–8,73	8,73–9,30	9,30–9,87	9,87–10,45	10,45–11,04	11,04–11,61	11,61–12,18	12,18–12,75	12,75–13,32	13,32–13,90	13,90–14,48	14,48–15,05	Number of fibres measured
31	8	2										2373
12	2											2405
30	8	1										2300
201	198	180	139	117	53	[illegible]5	10	8				2678
267	274	229	115	41	11	5						3224
227	195	179	129	106	58	31	8	4	1			26[illegible]
114	110	106	101	65	27	7	3					1968
216	166	121	64	28	12	5	2					3074
112	101	54	26	21	18	13	7	3	1			1487
210	204	134	126	80	38	17	14	3				2508
282	244	142	109	72	27	15	4	0	1			3035
149	139	131	104	51	34	9	2	1	1			2094
213	200	155	109	70	20	6	3	2				2197
178	160	121	94	40	15	5	2					1940
295	212	121	81	54	27	12	6	2				3282

VII TABLES

Tabk Ia Fibre distribution in sciatic nerves of normal r ts 5—40 days old

Age in days	Animal nr	0,42–0,80–	0,80–1 19	1 19–1,57	1,57–1 96	1 96–2,33	2,33–2,72	2,72–3,10	3 10–3 49	3 49–3,87	4
5	244/1	4	48	173	644	474	229	110	38	6	
	244/2	4	42	228	561	389	190	62	17	4	
10	289/2	0	0	17	279	373	204	143	98	80	
	289/5	0	0	19	233	416	251	236	151	86	6
	289/7	0	0	44	485	490	284	185	96	50	
15	222/3	0	2	68	428	360	287	290	262	253	16
	222/4	0	0	18	202	274	13	280	273	269	194
	222/5	0	1	115	521	282	218	245	208	192	179
20	221/4	0	4	17	72	04	215	240	298	305	25
	221/5	0	8	40	87	211	282	287	236	329	261
	221/6	0	1	29	140	302	402	423	433	362	253
25	223/5	0	6	33	129	224	166	191	257	264	276
	223/8	0	0	26	109	212	139	144	139	190	191
30	213/5	2	6	11	77	278	264	238	221	11	229
	213/6	1	12	31	133	225	183	149	146	168	24
	213/7	0	1	18	103	353	305	268	232	248	277
40	222/2	0	0	6	77	127	121	122	126	126	133
	/6	0	0	1	111	99	85	86	134	120	163
	(7	0	0	17	91	95	98	113	164	127	120

Fibre distribution i sciatic nerves f normal at 60–180 day ld

ge in days	Animal n	1 26–1 83	1 83–2,4	2,42–.98	2,98–3,56	3,56 4 14	4 14–4 70	4 70–5 28	5,28–5 86	5,86–6 43	6 43–7 01	7–7
60	221/3	8	194	274	274	231	216	185	221	235	245	162
	221/7	6	201	241	230	217	278	293	324	263	167	99
	221/8	4	169	217	249	227	250	242	250	226	216	137
90	200/1	11	58	113	146	162	136	180	151	171	172	218
	200/2	11	44	127	191	237	200	196	14	234	257	315
	200/3	14	42	78	162	167	154	168	139	157	214	219
120	139/2	9	94	121	123	139	133	115	137	122	137	147
	139/6	15	101	174	222	.51	271	210	209	225	250	251
	215/5	0	6	22	57	121	146	137	145	129	111	121
150	243/1	4	85	115	117	174	163	164	168	148	145	186
	243/2	4	115	161	142	230	175	171	179	205	179	281
	243/3	1	56	87	1 4	148	147	161	154	148	148	147
180	245A/2	3	25	100	115	144	134	125	135	119	154	175
	45A/6	3	27	92	134	135	109	115	125	116	140	153
	245A/7	10	64	170	221	245	229	232	258	256	.54	265

[illegible]	[illegible]–5,73	[illegible]–6,15	6,[illegible]–6,[illegible]	6,[illegible]–[illegible],93	6,9[illegible]–[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	9,23–9,[illegible]	[illegible] of fibres measured
												1[illegible]33
												1144
												1[illegible]
												1[illegible]15
												1[illegible]79
												1[illegible]5
[illegible]	6	5										1565
9	27	4	1	1	1							1[illegible]44
[illegible]	0	4										19[illegible]5
3												[illegible]4
	1											924
[illegible]	48	[illegible]	[illegible]	9	6	1						1[illegible]3
[illegible]	17	[illegible]	49	[illegible]	4	4	1					985
9	6	[illegible]	63	35	—	[illegible]		2	1			1[illegible]3
[illegible]	53	4	37	—		1						1578
[illegible]	78	55	[illegible]	31	11							1[illegible]
[illegible]	91	[illegible]	81	43	33	13	9	1				2022
[illegible]	[illegible]	9	11	[illegible]	[illegible]	[illegible]	[illegible]	4	1			1562
149	132	95	10	100	1	[illegible]	[illegible]	3	1	4	5	2343
[illegible]	103	135	[illegible]	[illegible]	9	[illegible]	44	25	14	9	3	2019

8,16–8,73	8,73–9,30	9,30–9,87	9,87–10,45	10,45–11,04	11,04–11,61	11,61–12,18	1[illegible],18–12,75	12,75–13,32	13,32–13,90	Number of fibres measured
2										2110
19	5									2091
28	7									1542
41	67	31	13	5						2197
45	64	35	16	1						2379
122	87	50	20	8	1					1904
125	92	60	26	9	5					1868
129	102	74	35	18	9	9	5	3		2865
116	112	93	64	48	12	8	4	2		1977
90	59	41	79	18	8	2	1			1868
53	37	27	16	5	1					1636
117	105	72	45	17	6	4	0	1		2094
79	64	50	36	10	4	2	1	1		1338
65	51	46	16	7	1	0	1			1341

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 407

Physiology of Swimming Man

BY

INGVAR HOLMÉR

STOCKHOLM 1974

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 40

From the Department of Physiology, Gymnastik- och idrottshögskolan, Stockholm, Sweden
and
the Work Physiology Division, National Board of Occupational Safety and Health,
Stockholm, Sweden

Physiology of Swimming Man

BY

INGVAR HOLMÉR

STOCKHOLM 1974

Why is it easier to swim in the sea than in rivers? Is it because the swimmer swims by continually supporting himself against the water and we can gain more support in water which contains more solid matter? For sea water contains more solid matter than river water For it is denser and more capable of offering resistance

Aristotle (384 322 B C) Problems XXIII 13

Why do extremities become most chilled? Is it because they are so narrow? The channels in them because they are narrow have but little blood so that they contain but little heat for blood is hot

Aristotle (384 322 B C) Problems VIII.5



Fig. 1 Determination of oxygen uptake during swimming in the swimming flume

CONTENTS

INTRODUCTION 9

METHODOLOGICAL CONSIDERATIONS 11

- Abbreviations and symbols 11
- Subjects 12
- Statistics 12
- Methods 14
- Procedure 16

RESULTS AND DISCUSSION 18

- Mechanical load factors 18
- Metabolic and cardio-respiratory responses to swimming 21
  - *Energy metabolism* 21
  - Swimming versus running 22
  - Respiration 24
  - Circulation 27
  - Effects of water at different temperatures 32
  - Local factors 33
- Heat exchange with the environment 34
- Energy output and efficiency of different styles and strokes 38
- Concluding remarks 43

SUMMARY 45

ACKNOWLEDGEMENTS 48

REFERENCES 49

Physiology of Swimming Man

AKADEMISK AVHANDLING
som med vederbörligt tillstånd av matematisk-naturvetenskapliga fakulteten
vid Stockholms universitet för avläggande av filosofie doktorsexamen kommer att
offentligen försvaras i Wenner-Grens Instituts föreläsningssal,
fredagen den 19 april 1974 kl. 10.00

AV

INGVAR HOLMÉR
Fil. kand.

STOCKHOLM 1974

The present publication is based on studies reported in the following paper

I Holmér I Oxygen uptake during swimming in man J Appl Physiol 33(4) 502 509 1972

II Holmé I and P -O Åstrand Swimming training and maximal oxygen ptake J Appl Physiol 33(4) 510-513 1972

III Holmé I A Lundin and B O Eriksson Maximum oxygen ptake during swimming and running by lite swimmers J Appl Physiol 1974 (in press)

IV Holmé I E M Stein B Saltin B Ekblom and P -O Åstrand Hemodynamic and respiratory responses compared in swimming and running J Appl Phy iol 1974 (in press)

V Döbeln W von and I H lmér Body composition sinking force and oxyg n uptake of man during wate treading J Appl Physiol 1974 (in pre s)

VI Holmé I Mechanical efficiency of breaststroke and freestyle swimming (for publicatio 1974)

VII Holmé I Aerobi energy output and efficie cy of diff rent competitiv wimming strokes (for publication in 1974)

VIII Nadel E R I H lmér U Bergh P O Åstrand and J A J Stolwijk Energy exchang s f swimming man J Appl Physiol 1974 (in pre)

IX H lmé I and U Bergh Metaboli and thermal re ponse to swimming i wate at varying temperatures (for publication in 1974)

I the text these pape will be referred to by Roma figures I IX

A number of fundamental criteria must be considered in ord r to obtain reliable measures of the body s reaction to a given rate of work (i) Measurements must be made during work (ii) Measurements shall be made where applicable in steady-state conditions i e when various physiological functions have adapted to the load (iii) The work procedure during measurements must coincide with the natural exercise to the greatest possible ext nt (iv) It hall be possible to make an accurate determination and reproduction of the rate of work

Different types of rgometers have been designed for different types of work in order to satisfy these criteria

Up to 1970 n most of the studie made on swimming the emphasis has been on a determination of oxygen uptake heart rate and respiratory function Mea surements in water and a swimmer movements and propuls on entail complications which have prevented complicated studie

Swimming in a pool or a lake creates difficulties in reproducing swimming speed and the turns in a pool introduce another disturb ng factor Tethered swimming result in hydromechanical conditions which differ from those in free swimming clearly reducing the applicability of obtained data to free swimming

The first sci ntific thesis on swimming was written by DuBois Reymond (1905) who calculated nergy metabolism during swimming on the basis of measurements from other work forms and a numbe of mechanical considerations However the e data were criticized by Liljestrand & Stenstrom (1919) who measured oxyg n ptake during swimming in a lake Since that time a number of studie have been made using different experimental designs and method The duration of work in some of th latter studies was too brief i e 1 2 min to permit any measurement of energy requirements in steady state conditions (Karpovich & Millman 1944 Goodwin & Cumming 1966 Adrian et al 1966) In other studi s breath-holding during swimming in combination with the determination of oxygen uptake during the recovery phase following submaximal (Karpovich & Millman 1944) and maximal work (Karpovich & Millman 1944 Adrian et al 1966 Kli - sourus 1968) was used in order to stimate nergy requirements This technique ha been strongly questioned (Christensen et al 1960) Oxyg n debt s probab ly used to pay off more than the energy debt incurred by anaerobic processes (cf Åstrand & Rodahl 1970) Furthermore the nergy expenditure required to eli minate lactate formed during exercise i eventually twice the nergy yi lded from the breakdown of glycogen into lactat (Krebs 1964)

A numbe of workers hav also measured oxygen uptak in steady stat condition during swimming in th sea (Pugh & Edholm 1955) and in a pool (Fredrik-

sen 1945 Hemmingsen 1957 Andersen 1960 Åstrand & Saltin 1961 b Åstrand et al 1963 Klissouras 1968 McArdle et al 1971)

None of these swimming studies were able to satisfy the four criteria specified at the beginning of this paper Therefore the results obtained in these studies have limited validity (also see the comments in I IX) Despite more or less serious shortcomings in the methods used the cited studies have still managed to obtain some information which has proved to possess general valid ity (i) Trained swimmers are distinguished by a lower oxygen uptake at a given submaximal speed than untrained swimmers (ii) The freestyle (front crawl) and backstroke are more economical swimming style than the breaststroke and butter fly at any given speed (iii) Leg kicks are less efficient in the freestyle than arm strokes alone (v) *The highest values for pulmonary ventilation and heart rate* during maximal swimming are lower than in maximal running cycling and walking

The development of a swimming fl me (Åstrand & Englesson 1972) made it possible to reproduce swimming speeds and eliminated a number of technical problems associated with mea urement The four specified criteria could be satisfied by the present investigation and a relatively detailed analysis of the physiological response to swimming was performed

The purpose of the present study was thus to

devise and investigate methods for the determination of different variables during swimming in a swimming flume;

study circulatory and respiratory f nctions during swimming

compare physiolog cal response during swimming with the corresponding reactions during running and cycling

examine the manner in which the body s heat balance and oxygen uptake are affected by work in water at varying temperatures

determine oxygen uptake during swimming at different speed and with different swimming styles used by swimmers of varying capability and in varying states of physical condition;

analy e me h nical work during swimming and calculate m chanical fficiency

It sho ld be emphasized that the special problems related to und rwater swimming were n t considered

Abbreviations and symbols

Th abbreviati ns and symbols in this work with a few minor exceptions are applied according to the guidelines devised by the Glossary Committee of th International Union of Physiological Sciences (See Glossary Committee of the IUPS 1973 a b)

Abbreviation or Symbol	Term (un t)
a-v difference	arterio-venous difference
C	convective heat transfer ($W\ m^{2}$)
^{o}C	degree Celsius
ERV	expiratory reserve volume (l)
h_c	Convective heat transfer coefficient ($W\ m^{-2}\ ^{o}C^{-1}$)
IRV	inspiratory reserve volume (l)
J	Joule
kp	kilopond (=9 81 N)
$kpm\ min^{1}$	kilopondmeter per minut (=0 163 W)
$l\ min^{1}$	liter per minute
M	metabolic free energy production (W)
$m\ s^{1}$	meter per second
mm Hg	millimeter of mercury column (= 1 Torr)
N	Newton
	number of subjects determinations et
η	work efficiency (%)
P	pr ssure (mm Hg)
P_{aO_2}	arterial oxygen pressure
$\dot{Q}$	cardiac output ($l\ min^{1}$)
R	respiratory quoti nt (also RQ)
	correlation coefficient
RML	mean respiratory level (l)
RV	residual volume (l)
S_{aO_2}	arterial oxygen saturation (%)
SD	standard deviation
SE	standard error of th mean
SMR	standard metabolic rate (W)
T	esophageal temperature (^{o}C)
T_m	m scle temperature (^{o}C)
$\bar{T}_k$	mea skin temperature (^{o}C)
T_w	water temperature (^{o}C)

TLC	total lung capacity (l)
V_E	pulmonary ventilation ($l\ min^{-1}$)
$V_E\ VO_2$	ventilatory equivalent
VO_2	oxygen uptake ($l\ min^{-1}$)
V_T	t dal volum (l)
\	Watt ($l\ J\ s^{-1}$)
W	rate of work (W)
W_F	sinking force of an immersed body (kp)
W_W	weight n water (kg)
<	less than

Subjects

Women and men of varying proficiency and varying degrees of swim training were used as subje ts Tabl I provides a brief description of the groups of subjects in the different studies (See each respective study for a more detailed description) A th Table shows th ir maximal oxyg n uptake was usually high in relation to the g eral population (Åstrand 1952 Åstrand 1960) and most of the subjects e w ll trained This made it possible to perform a number of trials at diff rent speed at each se ion including two maximal efforts in one day in some cases witho t any significant change in measured values as compared to trials on d ff re t day

All subjects were volu tee s and trained prior to th actual measurements so that they w e famil a with the equ pment and with th experimental designs

Stat stic

Conv nt onal ethods ere used the statistical processing in order to determine D E t ea diff rence and the t test (Armitage 1970) Calculation of m l ult ple reg s ion were pe formed using the least squares method co put j m BMDO2R (Health and Science Comput Facilities UCLA) wa d f s pwis m lt pl egression The error of the method has been pec f l t standa d deviatio fo a single experiment calculated from double d er at ord ng t the f llo ing equation

$$\text{Error of me} \quad l \quad \sqrt{l \quad \frac{\sum (d \quad d)}{n \quad l}}$$

In which d t iff c betwe double values $\bar{d}$ = the mean difference and n = the numb i t s

Table 1 Characteri tic of subject groups in the vario s studies Mean values and ranges are given Mi ed gro p refers to ubjects of varying skill and training status in swimming

Study		Age yrs	Height cm	Weight kg	Maximal oxygen ptake l min^{-1} Swimming	Running	Comments
I	3	20	174	66	2 89	3 24	Mixed group
		19 22	162 181	57 73	2 28 3 63	2 51 3 61	
	8	25	180	74	4 14	4 67	Mixed gro p
		16–47	179–183	71 74	3 32 5 08	4 03 5 59	
	12	15	165	54	2 96	3 17	Trained swimmers
		13 18	145 174	33 64	1 59 3 76	1 81 3 76	
II	1 (L)	19	180	73	3 63	3 61	Identical twins;
	1 (S)	19	181	69	2 82	3 56	L = elite swimmer
III	11	17	170	66	3 42	3 64	Elite swimmers
		15 19	167 175	57 71	2 94 3 76	3 25 4 04	
	12	19	184	78	5 05	5 38	Elite swimmers
		16 23	175 192	69 85	4 04 5 93	4 73 6 42	
IV	5	25	181	73	3 79	4 54	Mixed group·
		18 29	176–185	62 69	3 08–4 50	3 69 5 57	all trained
V	11	17	169	62			Trained swimmers
		16–19	161 179	54 69			
	4	26	179	75			Trained swimmers
		25 29	174 182	66 87			
VI	3	19	181	76	4 74		Elite swimmers
		16–25	175–188	63 84	4 05 5 19		
VII	19	19	180	74	4 49		Elite swimmers
		15 26	170–189	56–89	3 38–5 74		
VIII	3	26	179	76	4 13		Trained swimmers
		24 26	174 182	66–87	3 89–4 55		
IX	5	21	178	69	3 72	4 27	Mixed group;
		14 29	165 184	52 78	3 09–4 19	3 24–4 73	all trained

Methods

In this section a summary is provided of the experimental methods used A more detailed description will be found in the separate reports

Swimming was performed in a swimming flume (Stenberg Flygt AB Solna Sweden) in which water could be made to circulate at speeds from 0 0 2 0 m s^{-1} (Åstrand & Englesson 1972) Reproduceability for a given water velocity amounted to 1 4% the variation in speed for the water which a swimmer normally came in contact with was less than 0 02 m s^{-1} The task of the subject was to remain on the spot by swimming (Fig 1 2)

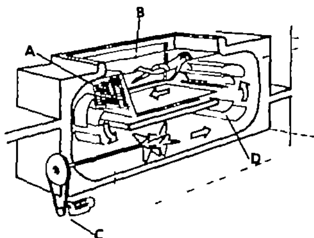


Fig 2 Drawing of the swimming flume A = safety net B = 2 5 x 1 5 m window; C = electric motor D = vane guided bends (from Åstrand & Englesson 1972)

Running took place on a motor driven treadmill whose speed and inclination could be adjusted Cycling was performed on an electrically or mechanically braked bicycle ergometer

Oxygen uptake was calculated from expired air samples collected in Douglas bags and analyzed for O_2 and CO_2 by the Haldane or the Scholländer techniq e after volume determination by bala ced spirometer Blood lactate level were de termined with the Barker S mmerson method as modified by Ström (1949) Cardiac output was established using the dye-dilution method with indocyanine green (Cardiogreen®) s the indicato (IV) Heart rate was calculated on the basis of ECG records Intra arterial blood pressure was measured with an Elema Schönan dor pressure transdu O_2 content O_2 saturation and CO_2 content of the blood

were det rmined using a van Slyke device while O_2 and CO_2 pressures and pH were established according to Astrup s technique (IV) Eosophageal temperature muscle temperature and skin temperature were determined with th rmocouples and heat flow was measured with Hatfield Turner heat flow discs (VIII IX) Mean skin t mperature and the average heat flow from the body were determined as a weighted mean value of four and five measurement points respectively on the body (VIII)

The body s sinking force and water drag were measured with a wire strain gauge and recorded using a Honeywell recorder (V) A method devised by di Prampero et al (1974) was applied in the determination of the body s drag during swimming (VI VIII) Mechanical efficiency was calculated as the ratio between mechanical work and the nergy metabolism during wo k min s standard metabolic rate (VI):

$$\eta = \frac{W}{M \quad SMR} \times 100\ (\%)$$

The weight of each subject in water with the body completely submerged the residual volume of the lungs and the body s dens ty were determined according to a method described by vo Döbeln (1956) (For detail on these and following anthropometric measurements see I and V) The weight of adipose ti sue and the averag thickn ss of subcutaneous fat were calculated on the basis of skinfold measureme t from the abdom and thigh (Hermansen & von Döbeln 1971) Skel tal w ight was calculated from mea urements of the width of the right and left femoral condyle the right and left radioulnar width and body height (von Döbeln 1959) Total potassium wa determined by means of whole body counting employing gamma spectrometry in a lead chamber (vo Döbel & Lindell 1964) The weight of fat free soft tissue wa calculated from the amount of pota sium

Det rmination of pulmonary volumes and certain pulmonary functions were made with the aid of a Bernst in spirometer (V)

Comm nts

In every case each se sor and the recording equipment were calibrated in con junction with each individual tri l session The erro of the method for a few mea sured variabl s shown Tabl 2 This includes both error of measurement and biological variat o s and wa calculated from two d terminatio s mad o different t st occas on or o the same (cardiac o tput) Th error of method fo determin-ati n of oxyg n ptake and cardiac utput were in close agreement with values re ported previously (Ekblom 1969 Kilbom 1971 Eriksson 1971)

Oxyg ptak val es stabli hed with and without arterial and v nous catheters during maximal swimming and running did not differ significantly (IV) Swimming i th swimming flume entailed prereq isit s for attaining a oxygen uptake as high a in swimming in a pool (Table 2 III)

Table 2 Error of the method in determination of oxygen uptake cardiac output and correlation coefficient and difference between means for some physiological variables measured under different conditions

	n	Mean value	Error of method	Difference between means	Correlation coefficient
Oxygen uptake (l min^{-1})					
Submaximal swimming	59	2 76	2 9		
Maximal swimming	47	3 78	2 1		
Cardiac output (l min ^{1})					
Submaximal swimming	11	17 3	5 4		
Maximal swimming	5	26 0	3 6		
Submaximal running	18	18 6	5 2		
Maximal running	7	28 4	4 2		
Drag (kp)					
Passive towing	61	3 51	3 9		
During swimming	6	2 92	5 7		
Oxyg n upt k (l min ^{1})					
during maximal swimming in flume vs pool	7			0 02	0 99
Oxygen ptak (l min ^{1})					
during swimming and running w th and without catheters	10			0 01	0 99
Arterial O saturation (%)					
with and without alve	12			1 6	0 89
Art rial O_2 pressure (mm Hg)					
th and without valv	17			−0 2	0 91

Procedure

Detail o th expe imental des gn in the different studies are reported in each respective art cle Only some general princ ples will be discussed i this section

At each test s ss o ork was eithe performed on a treadmill bicycle ergometer or in a swimmin t A a rule a series of determinations ere made at light moderate heav aximal intens ties Light moderat avy work int s ties The total duration of exercise at each submaximal inten s lly amounted to 5 7 min Heart rate was recorded each minute oxygen upta during the f nal 1 2 min and blood samples for lactate assay

were taken 1 2 min after the conclusion of exercise and occa ionally 5 10 min after exercise in order to guarantee attainment of peak values

Maximal work int nsities The maximal oxygen uptake specified for a subject generally consisted of a mean value for determinations mad at a few days interval In the determination of cardiac output and in conjunction with work i water at different temperatures the subjects maximal oxygen uptake was determined in trials prior to the start of the actual study In some exceptional cases there was no opport n ity of obtaining two independent determinations of a subject s maximal oxygen uptake and the following procedure was then applied Maximal ru ning a constant speed was used and the inclination at the start was 3^{O} (5 25%) being increased by 1 5^{O} (2 60%) every third minute Maximal swimming water peed was increased by 0 1 m s^{1} every oth minut starting with a speed which was 10 20% lower than the maximal speed calculated for each subject (III) I both modes of exe cise work was performed until complete exhaustion

Comments

The average differe ce in oxygen uptake (0 02 1 min^{1}) between two determinations mad with 19 subject who performed maximal swimming at an interval of few days wa not significant The difference in swimming speed amo nted to 0 08 m s^{1} (7 7%; rang 4 4 20 0%) The duration of exercise for th test at a somewhat lower speed wa 5 27 min (range 3 35 7 00) and for the t st at a somewhat higher speed 3:23 min (range 2:20 4 40) Thus maximal oxyg ptake co ld be a hieved with a work duration of 3 4 min o 5 7 min during swimming (f Åstrand & Saltin 1961a). The levelling-off criterion for the determinatio of maximal oxyg n uptake was used as d scribed by Ekblom (1969) In this context th maximal swimming speed was stated as th lowest one at which th swimme achieved maximal oxyg uptake

RESULTS AND DISCUSSION

This section is divided into four parts the first of which provides a brief description of the mechanical load factors in swimming The second part deals mainly with the metabolic and cardio-respiratory responses to swimming in general The third part elucidates the heat exchange and the metabolic response to swimming in water at different temperatures The section is completed with a discussion of the various swimming styles from point of view of efficiency and maximal energy output and with concluding remarks

Mechanical load factors

A person with some part of his body in the water and with unimpeded airways is affected by a sinking force whose magnitude depends on a number of anthropometric variables (such as the weight of muscl s and adipose tissue) (Fig 3)

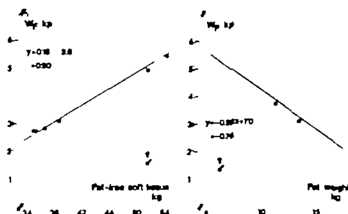


Fig 3 The body s sinking force in relation to fat free soft t ssue (left) and fat w ght (right) Regression line equation and correlation coefficient ntered (From V)

the w ight of body parts above th s rface of the water and the vol me of air in the lungs (Fig 4) If the lungs are filled with air to a maximum and the body is submerged so that only a small part is out of the water most people should be able to maintain a state of equilibrium in the water without the se of active m sclo work Du ing sw mming and water treading however a somewhat larger part of the body is normally t of the water and the lungs are only partly filled with air *Therefore the body s acted on by a sinking force* which must be counteracted with muscular force If the exercise is not very vigouro s (i e partly anaerobic) oxygen

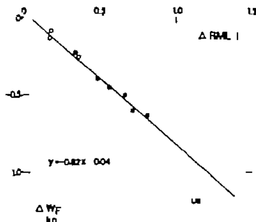


Fig 4 Change in sinking force i relation to change in respiratory mean level Regression line and equation ntered (From V)

ptake is the best measure of th extra energy yielded by th muscles

Oxygen uptak during water-treading proved to be directly related to the body s sinking force (Fig 5) (a relatio ship which was also described by Hemmingsen 1957) Individuals with a larg amount of body fat relatively speaking were acted on by a smaller sinking force than might be anticipated from body weight (Fig 3) This circumstance generally led to a somewhat higher body position in water for girls than for boys

An additional force compone t ari es during swimming since when a body moves through a fluid a force acts backwards on it resisting it motion This force is known a drag and its amount depend on the fl id and n the size shape

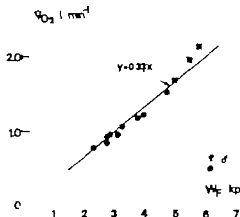


Fig 5 Oxyg n uptake during water treading in relatio to sink ing force Regre io line and equation nt red Th correlation coefficient wa 0 96 Wh W_F was increa ed by loading subjects with known weight the increas in $\dot{V}_{O_2}$ followed the regre ion line (From V)

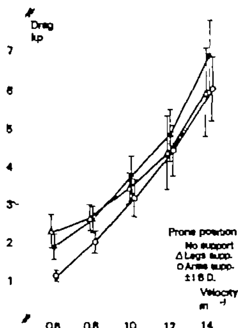


Fig 6 Drag during passive towing of the body in three positions in relation to water speed Mean value + 1 SD for seven male subjects (From VIII)

and speed of the body Individuals with a large body surface are exposed to greater water drag (Karpovich 1933 Alley 1952 Hansen 1955 Counsilman 1968) The body s *position in water and during the different phases of the swimming stroke* affects the amount of water drag (Counsilman 1955 Kent & Atha 1971)

Drag during passive towing of the body through flowing water increased exponentially as the water speed increased (Fig 6) At higher speeds the water exerted a lift on the lower parts of the body (hydrodynamic lift) and therefore the body assumed a more horizontal position at high speeds than at low speeds If the legs were lifted into a horizontal position using a cork plate at high speed drag was furth r reduced (Fig 6) When the subject was swimming drag in breaststroke and freestyle was calculated to be approximately 2 and 1 1/2 times greater respectively than in passive towing (Fig 7)

Thus total drag during swimming was greater than in passive towing Corresponding re ults hav been reported by other authors (di Prampero et al 1974)

* In this context stroke is defined as a complete work cycle of arms or legs or both Style refers to the type of swimming e g freestyle (front crawl) back stroke breaststroke or butterfly (dolphin stroke)

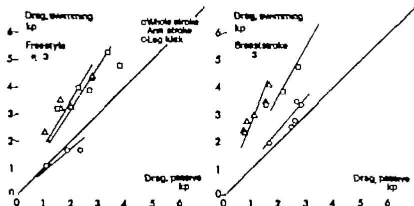


Fig 7 Drag during swimming with the whole stroke and with arm or legs alone in the freestyl (left) and breaststroke (right) a compared to passive drag at corresponding velocities (From VI.)

Since the lower part of th body is denser than the upper part there s a more pronounced tendency for the legs to be drawn down when the body as me a horizontal position in water In order to attain the most favourable po ition from the point of view of drag a certain amount of work must be performed with the legs to counteract this downward pull on th legs The s gnifica ce of thi force on the legs was not examined in detail in thi study and therefore not co sid red when calculating the mechanical work

Therefore mechanical work during swimming has be expre sed a water drag multiplied by the distance swam (VI) M chanical effici ncy ca be calculated by measuring energy metabolism during swimming and at rest

Metabolic and cardio-re piratory responses to swimming

Energy metaboli m

During swimming at low speed th measured oxyg n uptake was of th same magnitude as during water-treading (Fig 8) Interindividual differences in th body s sinking force were reasons fo the noticed variations i energy expenditure at low speeds Thu a better measure of the effici ncy of swimming wo k was obtained when oxyg pt ke wa corrected for body w ight in water (Fig 8) A similar conclusion was presented by Hemmingsen (1957) Water drag increases by about th square of the peed (V^2) and as mentioned a more horizo tal body positio is assumed at higher speed The result is that drag at high peeds constitute the main loading factor

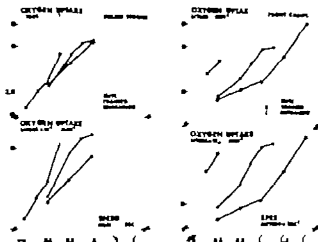


Fig 8 Oxygen uptake in relation to speed during breaststroke (left) and freestyle (front crawl) (right) swimming by three subjects selected so as to repre sent three training categories In the lower half of each graph oxygen uptake is corrected for subject weight in water (From I)

Oxygen uptake d splayed a linear increase or a slightly exponential increase as swimming speed increased (Fig 9) At the highest speeds oxygen uptake levelled off This means that a maximal oxygen uptake was achieved by each subject at a given speed and was not exceeded even if the swimming speed was increased somewhat (Fig 9)

The oxygen ptake at a given submaximal speed varied among subjects Some of these interindividual variations could be explained by differences in water drag (Fig 10)

The increase in oxygen uptake as submaximal speed increased was governed both by the increased drag as well as the swimming style and swimming technique depending partly on the degree of training A more detailed discussion of the signi ficance f these factors will follow

At a give submaximal oxygen uptake blood lactate tended to be lower in trained than in nt ained swimmers Trained swimmers were able to work at a relatively high oxygen uptake (60–70%) in relation to their maximum without any elevation in blood lactate (I VII)

Swimming versus running

An examination of the physiological response at a given oxygen uptake during swimming is of considerable interest as the exercise medium and the position of the

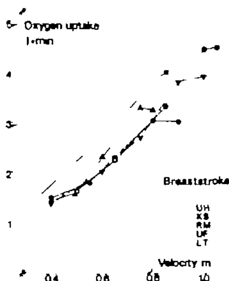
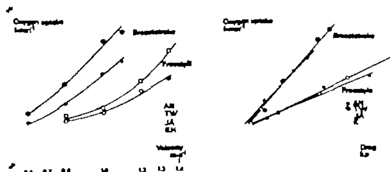


Fig 9 Oxygen uptake displayed linear or exponential increase as swimming speed increased and levelled off at higher speeds This levelling off was used with a f w exceptio s to designate maximal oxygen uptake but was not generally included in the graphs (From IV)

body is so different in this form of ex rcise than in e g running and cycling The present study made a detailed analys s of th circulatory and respiratory respo se during the breaststroke The following results were considered in selecting this swimming style

1 The oxyg n uptake attained in maximal breaststroke swimming was at least a great a i the other swimming style (I III VII)
2 Maximum load on the swimmer is achieved at a lower swimming speed than in othe swimming styles (VII)



F g 10 Oxygen uptak fo two equally succe sful breaststroke and freestyle swimmers respectively in relation to swimming peed (l ft) and pa i drag at corre ponding peeds (right) Note the individual diff rences wh n oxyg n ptake i related to velocity and the almost congru nt relatio ship obtained when related to body drag (From VII)

3 The swimmer is relatively easy to communicate and the style presents few problems regarding interference with the probes

Running and cycling were chosen for purposes of comparison

In previous studies maximal oxygen uptake in swimming in a pool was reported lower (Åstrand & Saltin 1961 b Åstrand et al 1963 McArdle et al 1971) but in one study higher (Magel & Faulkner 1967) than in other types of exercise The latter finding however is of limited value since swimming tests were administered later in the training season than running tests Elite swimmers attained in swimming a maximal oxygen uptake which was significantly lower (6–7%) than in running (Fig. 11 I III) but of about the same magnitude as in cycling (I) For untrained swimmers maximal oxygen uptake in swimming was reduced even more i e by about 20% compared with running and by about 10% compared with cycling (I) The explanation for the reduced maximal oxygen uptake in swimming must lie in limitations in the oxygen transport cha n

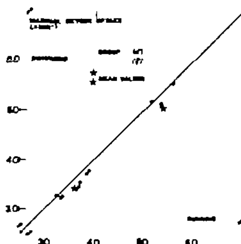


Fig 11 Comparison of maximal oxygen uptake during swimming and running by eleven female and twelve male elite swimmers (From III)

Re piration

For a itting subject the vital capacity is reduced by 8 10% when the body ex cept for th head is submerged in water (Agostini et al 1966 Hong et al 1969) Most of this reduction should be ascribable to an increased blood volume in the thorax (Hong et al 1969) The increased airway resistance during submersion at rest (Agostini et al 1966) and during swimming (Deroanne et al 1971) must en tail increased respiratory effort (Hong et al 1969)

Respiration in swimming is synchronized with swimming strokes and the duration of the inspiratory phase is reduced (except in the backstroke) Expiration usually takes place under the water surface (except in the backstroke) and accord

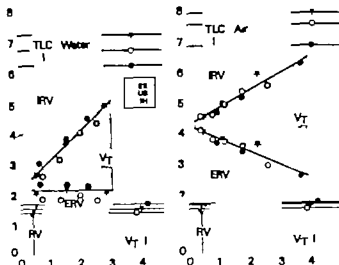


Fig 12 Total lung capacity in piratory reserve vol me residual vol me and tidal volume fo three subjects in air and in water Note that the mean respiratory level shifts towards the ERV in water and that the increase in V_T takes place exclusively by utilizing IRV (From V)

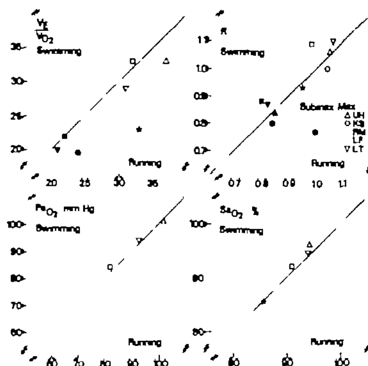
ingly against greater resistance than in air However in the present studies this was compensated by the use of a respiratory valve Impaired ve tilation may interfere with th transport of oxyg n from the atmosphere to the pulmonary capillaries

In water th i creased tidal volume was attained exclusively through the use f th inspiratory reserv volume in contrast to circumstances in air (Fig 12 V)

During submaximal swimming V_E $\dot{V}_E$ V_{O_2} and R were related to $\dot{V}_{O_2}$ in the same manner a in running and cycling (Fig 13 I IV)

During maximal swimming V_E and V_E $\dot{V}_{O_2}$ displayed mean values which were gnificantly lower than in maximal running and cycling (Figs 13 14 I III IV) Tidal volume during maximal swimming was of the same magnitude as in maximal running while respiratory rate apparently governed by the swimming stroke rate was lower during maximal swimming

Values for arterial O_2 saturatio O_2 and CO_2 contents and O_2 and CO_2 pressures during both submaximal and maximal work were the same in swimming and running (Fig 13 IV) Blood ga values tak n with and without the respiratory valve during maximal swimming wer almost ide tical (Fig 15) Alveolar v ntilation per breath



F g 13 Comparisons of ventilatory equival nt respiratory quoti nt arterial O_2 pressure and arterial O_2 saturation in swimming and running Submaximal swimming values interpolated (From IV)

calculated using a modified Bohr s formula was greater during both submaximal and maximal work in swimming than in running (IV)

It was obvio s that the pronounced hyperve tilation sually associated with maximal exercise on land did not develop in swimming Blood gas data how ever provided no indication of hypoventilation Th s the pulmonary ventilation ing maximal wimming was sufficient to saturate the blood with oxyge to the same degree as d ring ru ning Th respiration doe not appear to be restrict ive to the ma imal oxygen ptake h ch can be achieved in swimming and is no explanation of th lower maximal oxyg uptake noticed in swimming

It should b mphasized that a number of scientists have shown that oxygen p- take during submaximal and maximal cycle work is only influenced insignificantly even when respiratory resistance is doubled (Demedts & Anthonison 1973 Flook & Keelman 1973) Pulmonary ventilation however during maximal exercise was about 10% reduced (Demedts & Anthonisen 1973) Hence the ventilatory equivalent was lower during maximal cycling with increased external airway resistance This is also in agreement with the respiratory response to maximal swimming

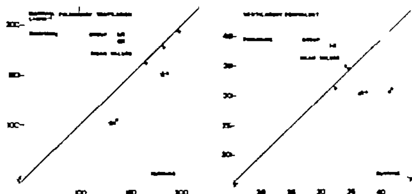


Fig 14 Comparison of maximal values for pulmonary ventilation and ventilatory equivalent in swimming and running by elite swimmers (From III)

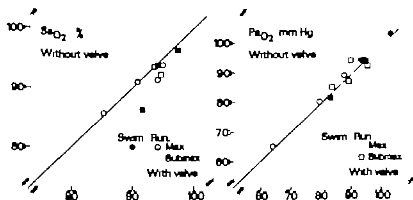


Fig 15 Arterial O_2 saturation and arterial O_2 pressure compared during swimming with and without respiratory valve (From IV)

<u>Circulation</u>

Circulation th next link in the oxygen transport chai ha been the subject f very few detailed studi s during swimming Lilj strand & Lindhard (1919) nd Dixo & Faulkner (1971) measured the cardiac output during tethered sw mming using the gas rebreathing technique Saltin (1973) has reported data on cardi c output during swimming but only fo one subject

Cardia output during swimming di played an almost linear increase s V_O in creased and at a given <u>submaximal</u> V_{O_2} it was about the same as in running (Fig 16)

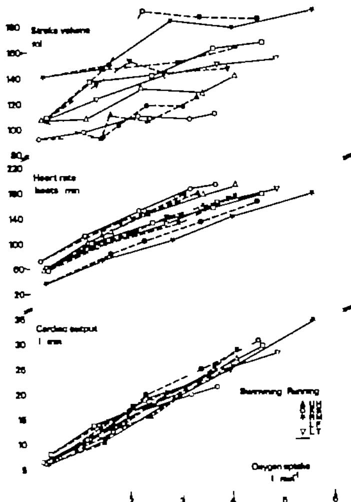


Fig 16 Stroke volume heart rate and cardiac output d ring swimming and running in relation to oxygen uptake (From IV)

Also heart rat during swimming displayed a linear increase related to VO_2 and was of about th same magnitude at a given submaximal VO_2 as in running (Fig 16)

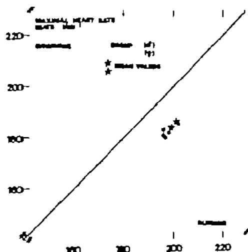
Cardiac output and heart rat during maximal swimming were less than in maxi mal running (F gs 17 18) The calculated stroke volume d ring submaximal and maximal swimming was of abo t the same magnitude as in running Stroke volume increased i both erci e forms i the transition from supine rest to light submaxi mal work thereafter remaining relatively constant as the rat of work increased (Fig 16)

The systemic a-v O_2 difference at a given submaximal $\dot{V}O_2$ was about the same

Fig 17 Val e for oxygen uptake heart rate cardiac output strok volume arterio-venous O_2 diff rence and mean blood pre sure compared during swimming and running Submaximal valu s compared at corresponding oxyg n uptake (From IV)

in running and swimming but lightly lowe in maximal swimming than in running (Fig 17) Howeve oxyg n utilization in the working legs appears to have been at least as great in swimming a in running a the a v O_2 difference in working legs wa similar (IV) Thi discrepancy n oxygen extractio between swimming and running must reflect differences in distribution of blood flow implicating a relatively large blood flow i less oxygen consuming tissue g intestinal organs and skin in swimming In short term maximal exercise n land priority is given to muscl blood flow which th running data in thi study confirm Data are needed on regional blood flow in swimming to explain accurat ly the observed differences between systemic and leg a-v O_2 difference

Th cardiac utput can be limited by both central factors such as heart rate and stroke vol me and by peripheral factors such as the size of the va cular bed influencing th peripheral resistance and th perfu ion pre sure As mentioned i the introductio a heart rate signif cantly lower i maximal swimming than in maximal running cycling and walking i a well-documented phenomenon (cf McArdl t al 1971) (Fig 18) It is also a well known fact that face immersio i combi ation with breath holding will lead to a depres ed heart rate (diving bradycardi)



F g 18 Maximal heart rate com pared during swimming and running by el te swimmers (From III)

and this reduction is more pronounced th colder the water (Andersen 1966 Strauss 1970 Moore et al 197) Diving bradycardia has also been demonstrated during exercise (Scholande et al 1962 Asmussen & Kristiansson 1968 Strömme et al 1970 Bergman t al 1972) However breath holding appears to be the most mportant cause (Brick 1966 Asmussen & Kristiansson 1968 Paulev 1968 Strömme et al 1970) Thi is why criteria during swimming fail to fully satisfy the criteria assoc ated with diving bradycardia But the effect of a diving reflex may persist Th s hypoth s upported as the heart rate i somewhat lower during swimming w th face mme ion than in swimming with the head out of the water at a corre ponding oxygen ptake (Asmussen & Kristiansson 1968)

Different body pos tions during work i e whether the work is performed in an upright or recumb nt position has also been shown to influ nce circulation The improved conditio s for heart f lling during diastole in a recumbent position may expl the somewhat higher stroke volume at rest and at a lighter submaximal oxygen ptak a compared with sitting rest and work respectively (Bevegård et al 1963 t b rg t al 1967) However stroke volume during maximal work is the same d ring leg cy ling nd comb ned arm and leg cycling irrespective of body pos ti n (St nbe g et al 1967) a finding in agreement with the present results i ma mal wimming and running However recumbent cycling is in a way an unnatural fo m of x s Swimming on the other hand normally takes place with the body n a ho i o tal positio l

During subm r ion i water th hydro tatic pressure counteracts the inspiratory work and in swimming m ntioned expiration normally takes place in the water It is shown that po iti p ss e breath ng i man at supine rest in air entails a reduced cardiac output and that this reductio indicates an impaired venous return

which follows the increased intrathoracic pressure (Werkö 1947) However Arborelli s et al (1972) demonstrated a 30% increase in cardiac output in man submerged in water (35°C) compared with resting conditions in air Rennie et al (1971) have shown that in water below 34°C cardiac output at rest and during exercise is lowe than at corresponding oxygen uptake in air probably d e to cold stress H nce in this respect the effects of hydro tatic pressure and cold wate respectively are similar

The large heat transfer coefficient between the skin and water (see the follow ing section) facilitates th heat transfer to the water thereby reducing the need for skin circulation Variou demands on skin circulation due to changes in skin temperature modify the circulatory response to a given submaximal work (Rowell et al 1969) A more d tailed discussion on these factors will follow

It is likely that the muscl mass activated in swimming although with both arms and leg is smaller than in running Th need for stabilization of the body in water i el ght so that work for this purpose is largely eliminated A smaller mus l mass involved in work means that a small r quantity of oxygen can be con sumed and therefore transported by the blood Thus the smaller cardia output and lower heart rat in maximal swimming compared with maximal running can be explained to some extent by a smaller oxygen uptake This hould be the main reason why particularly untrained swimmers had a lower maximal oxygen ptake in swimming than in running (I)

Th mean arterial blood pressure at submaximal and maximal rates of work was higher in swimming than in running (Fig 15) The highe mea arterial blood pressure i swimming can be explained by e g elevated external pressure and n creased peripheral resistance ca sed by the lower temperature (26 28°C) of the ki The difference in blood pressure may also be a result of a varying size of th activated muscle mas Stenberg et al (1967) showed that work at a g v n submaximal oxyg n uptake with small mu cle groups produced higher blood pres ure than work with large muscle gro ps

The difference between an upright and a recumbent work position also compris differences in perfusion pres ure in working leg depending o the ffect of hydrostatic pressure (Folkow t al 1971) Blood perfusion therefore facilitated in running as compared with swimming However th levated art rial blood pre ure during swimming may result in a somewhat increased perfu ion (IV) But th importance of a ra ed perfu ion pressure also d pend on the veno pre sure At present there are no data availabl elucidating d ffe ence between work form in this re pect Thi circumstance pl s differences in body position and exercise media make it difficult to interprete land ex rcise data in term of swimming

The circulatory response to exercise in cold water may reflect a conflict between metabolic and heat regulatory requirements which have to be satisfied simultaneously by the cardiac output The result may be that neither of these requirements are adequately satisfied thereby impairing optimum function

Severe cold loading leads to a reduced blood flow in peripheral tissues (cf Keatinge 1969) which could jeopardize muscl blood flow The reduced maximal oxygen uptake observed when body temperature was lowered ($T_{es} < 37^{\circ}C$) (VII IX) may be one effect of s ch a mechanism

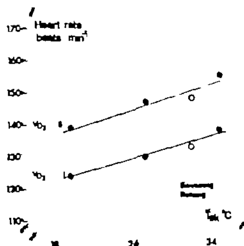


Fig 19 Heart rate in relation to mean skin temperature at two meta bolic rates Heart rate values inter polated (from IX) and $\bar{T}_{sk}$ calculated from VIII (swimming) and from Saltin et al (1972) (running)

The lower heart rate during submaximal and maximal swimming in the case of low central temperatures indicates a smaller cardiac output assuming stroke volume is unchanged Rowell et al (1969) showed that cardiac output and arterial pressure at given submaximal oxygen uptake varied in a similar manner when skin temperature changed Nielsen (1969) calculated skin blood flow at approximately 1 l min $^{-1}$ when V_{O_2} = 2 l min $^{-1}$ and T_{sk} = 33°C An increase in cardiac output from 15 l min $^{-1}$ to 16 l mi $^{-1}$ with a constant stroke volume (140 ml) would then correspond to a 7 beats mir $^{-1}$ change in heart rate a value of the same magnitude as the difference in swimming at 18°C and 26°C and 26°C and 34°C respectively (Fig 19) The difference in peripheral circulation depending on various heat balance conditio s can explain som of the variation in heart rate during exercise in water at varyi g t mperatures and the difference in heart rate between maximal swimming and maximal running (Fig 20 IX)

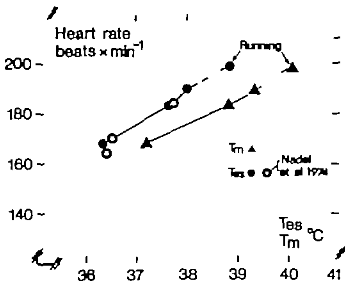


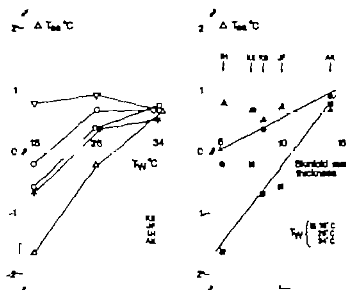
Fig 20 Heart rat during maximal swimming and maximal running in relation to sophageal temperature and m scle temperature Mean values for five mal subjects Data from VIII ltered (Nadel t al 1974) (From IX)

A numbe of physical and ch mical processe in the body are thermosens tiv Thus the blood s viscosity increases as it temperature drops and the rate of diffusion and enzyme ctivity decline When the temperature of the blood drops a mall r volume of oxygen is give ff from the hemoglobin at a g v n oxygen pre sure (the Bohr effect) In maximal work however oxyge extraction locally in working muscl s should be almost maximal thereby independent of variations in th oxyg binding capacity of hemoglobin due to smalle change in blood tempera ture In conclusion the factors mentioned may aggrevate the distribution of oxyge to working m scles and the mitochondrial oxidation

Local facto

Limiting factors in the oxyg transport chain are also linked t the gas exchang between capillarie and m scle cells and to properties of the muscl cell and its environment Gollnick et al (1972) found in five elite wimmers a high percentag of low twitch fibers in shoulder mu les (average 75%) whereas the proporti thigh musculatur was equal to or somewhat l s than the valu found in athlet in other endurance ports (average 60%) Myoglobin is suggested to facilitate oxygen utilizatio by enhancing oxygen transport through th cytoplasm M sal myoglobin content is increased (Whipple 1926 Pattengale & H lloszy 1967) and

Fig 22 Change in T_{es} after 20 min of submaximal swimming in water at three different temperatures (left) In the right panel this change in T_{es} is related to subject skinfold thickness Regression line drawn for each temperature (From IX)



Heat production certainly increases in conjunction with muscular activity e g as in shivering or when exercising The low degree of efficiency in swimming (see the following section) means that 90–95% of the energy output degenerates into heat However both shivering and swimming increase the tissue conductance (Burton & Bazett 1936 Edwards 1950) leading in cold water to the emission of more heat than is produced during work Therefore the body is cooled more rapidly than would be the case if the body were kept inactive (Keatinge 1961)

In water at 18°C and 26°C no accelerated cooling was noted in our subjects (VIII IX) Mean skin conductance was relatively independent of swimming speed

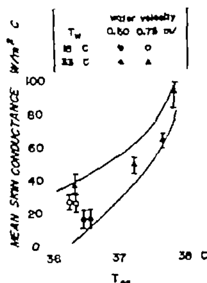


Fig 23 Mean skin conductance in relation to T_{es} during swimming in water at two different water temperatures Mean values for three subjects (From VIII)

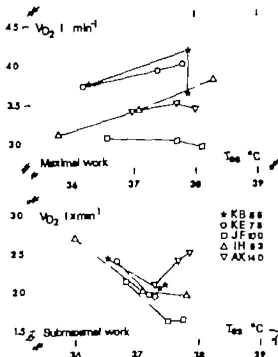


Fig 24 Oxyge uptake measured during submaximal (lowe panel) and maximal swimming (upper panel) in relation to T_{es} Values fo subject skinfold thicknes are entered (From IX)

sugg sting that i this temperature range elevated heat production caused by work was beneficial to reduce the rate of body cooling In warm water skin conductance increa ed sharply as swimming speed increa ed thereby preventing rap d storage of heat in the body (Fig 23)

With a low internal body temperature (T_e < 37°C) oxyg n ptak at a giv n submaximal peed wa raised by about 0 5 l min⁻¹ and the high st oxygen p-tak wh ch could be achieved by thin subjects wa 6–18% l s tha at a normal or ra sed T_{es} (Fig 24) It should be emphasized that the elevated oxyge ptake n submaximal swimming plus th red ced maximal oxygen uptak severely restrict swimmer performance and reduce endurance

To summarize person with a thick layer of subcutaneou fat are better equipped than thin persons to withstand cooling in cold wat Th rate of cooling can be influenced to som ext nt by increased heat prod ction through voluntary exerci o shivering and reduced peripheral circulation In water at 18 26°C the benefit of

increased heat production during exercise in most people outweighs the liability of the increase in convective heat loss achieved in conjunction with vigorous swimming that accompanies the increased heat loss (cf Craig & Dvorak 1968) However our data suggest that very thin persons may be exposed to an accelerated cooling even in water at 18 20°C in conjunction with increased work Rest in still water results in a lower level of heat production but also in lower values for skin conductance than in swimming and may therefore be an equally good means of avoiding severe cooling It should be noted that clothing reduces skin to water heat loss (Keatinge 1961)

Energy output and efficiency of different styles and strokes

The results in this section are primarily based on studies I II III VI and VII

The efficiency of propulsion in water depends to a great extent on the swimming style This is readily apparent n a glance at existing wo ld records (Table 3)

Table 3 World records for men swimming 200 m (1973) Mean swimming velocity and the percentage of freestyle swimming speed are shown

	Time min	Velocity $m\ s^{-1}$	%
Breaststroke	2 19 28	1 44	81
Freestyle	1 52 80	1 77	100
Backstrok	2 01 87	1 64	93
Butt fly	2 00 70	1 66	94

F g 25 show mean values fo oxygen uptake for lite swimmers swimming diffo ent styles Oxyg n uptake was 1 2 l min^{-1} less in the freestyle and back stroke tha in th b eaststroke and b tterfly The maximal swimming speed in the freestyle and ba k troke was about 0 25 $m\ s^{-1}$ faster tha in the other two swim ming stylo Mea ma imal oxygen uptake by elite swimmers amounted to 4 6–4 9 l min^{-1} th ba kstroke breaststroke nd butterfly (Fig 25) The somewhat lower ma imal o gen uptake in th freestyle in this study (4 3 l min^{-1}) can be explained by the f ct that this section also comprised a few less well trained swimmers

The results shown in Fig 25 are in good agreement with world records with the exception of the b ttorfly It h ld be not d that the work durations for existing world records were about 2 mi whereas the swimming reported in Fig 25 lasted 5–6 min In two minutes f maximal work work capability is governed by anaerobic

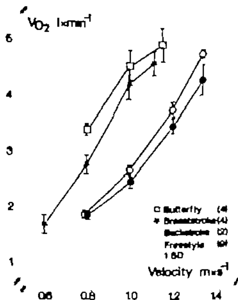


Fig 25 Mean values for oxygen uptake in relation to swimming speed by male elite swimmers in the four competitive swimming styles Numbe of swimmers i denoted within parante sis Oxygen ptake levelled off when speed was further increased (not included in th graph) (From VII)

power to a greater extent than in 5 6 min of work in which maximal aerobic power is a limiting facto

Th breaststroke and butterfly provide somewhat unfavourable mechanical conditions for propul ion in wat r I the breaststrok both the arms and legs are recovered in the water leading to high drag values Arm recovery in the butterfly makes it necessary to lift part of the upper body out of the water This lift probably consumes a great deal of energy and make t impossibl to maintain a high speed for any great length of time

Oxygen uptake in the freestyle and backstroke appears to display n exponenti l increase s swimming speed increase whereas th increase of oxygen ptake during th breaststroke and butterfly was linear or somewhat less at high speed (Fig 25) Thi may be because the swimming strokes in both th se styles are symmetrical re ulting in great r acceleration in propulsive phase so as to compensate fo th retardation n non-propulsive phase than is the case in th mor continuous propulsion in the freestyle and backstroke Th refore efficiency probably increas s when th rat of swimming stroke increas s at higher peed

Efficiency in swimming wa poor (4 7%) a compared to g cycling (20- 2%) but of the same magnitud a reported by di Prampero et al (1974) using a similar measuring technique The freestyl displayed greate effici ncy (6 7%) tha the breaststrok (4 6%) An untrained swimmer displayed an effici ncy of 2 7% in th breaststroke (VI)

The greatest efficiency (7 7%) wa measured in the freestyle ing only th arms

whereas leg kicks in both the breaststroke and the freestyle displayed poor efficiency (2–4% and 1 2% respectively) (VI)

From the phy iological point of view maximal performance in swimming is mainly governed by two factors viz the maximal power of energy yielding processes and the degree of efficiency (swimming technique)

Elite swimmers especially in distance events are distinguished by high maximal aerobic power (I III VII) Maximal oxygen uptake for twelve male swimmers was found to be 5 05 l min ^{1} (range 4 04 5 93) in swimming and 5 38 l min ^{1} (4 73–6 42) in running The corresponding values for female swimmers were 3 42 l min ^{1} (2 94–3 73) and 3 64 l min ^{1} (3 35–4 04) respectively In relation to body weight maximal oxygen uptake in running amounted to 68 6 ml kg ^{1} min ^{1} (62 5 76 4) for male sw mmers and 55 3 ml kg ^{1} min^{-1} (47 8–61 2) for the female swimmers (III)

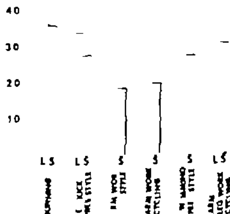


Fig 26 Oxygen ptake during var ous types of exerc se with maximal intensities fo 3 5 min Open symbol refe to the untra ned (S) and closed symbols to the el te swimmer (L)

Thes val e rere comparable to those obtained in other endurance events (Salti & Åstrand 1967 Ekblom & Hermansen 1968) Swimmers generally weigh more than e g runne which s why their maximal oxygen uptake values expressed in ml min ^{1} kg ^{1} re fa lower than for good runners In water body weight is by no means the same loading factor as in running (I IV) This suggests that a swimmer s maximal oxygen uptake sho ld be expressed in l min ^{1}

Oxyge uptake during maximal u ining uphill may be regarded as an absolute measure of an individual maximal a rob c power Elit swimmers as already mentioned displayed a 6 7% lower oxygen upt ke in maximal swimming Maximal oxygen uptake in untra ed swimmers was reduced by about 20% in swimming as compared to running (I) Th s training enhances physical performance but it is *important for this training to take place in water to the greatest possible extent*

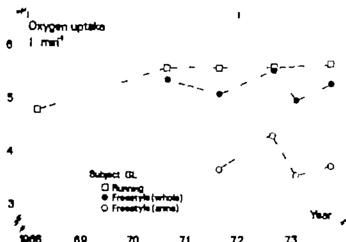


Fig 27 Oxygen uptake during swimming and running at maximal intensitie by one world-class swimmer over the past six years Note the unchanged oxyg n uptake during running from 1970 where a oxygen uptake during swimming with the whole stroke and with only the arms varied co siderably during the seasons (From Holmé 1973.)

Two sisters (identical twins) displayed the same maximal oxygen uptak in running but the one who participated in hard swim training displayed a 24–49% higher oxygen uptake in different swimming styl s than her sist r who wa not specially trained by swimming (Fig 26) (It hould be emphasized that both of the girls were taking part in othe inte sive physical training) Results from study II (Fig 26) therefore suggest that it is swim training which produces th main pe formance improvement in swimming

Fig 27 shows oxyge uptak in maximal swimming and maximal running by wo ld-clas swimmer during several year of intensive swim training Varying oxygen uptake valu were measured during maximal swimming owing to variatio s in training inte sity illness et Peak were noted during period in which the swimme was most successful The variation in oxyg uptake wa most pronoun ed in freestyl swimming using nly the arm (as me tioned earlier most important to propulsi n in the freestyle) It should be emphasi ed that running training wa only performed to very limited extent Oxyge ptake in maximal running wa still consist ntly higher than or equal to oxyg n uptake in maximal swimming

A poo swimming techniq by d finition explain why untrained swimmers at given speed equired a higher oxygen uptake than trained swimmers (I) A disting sh ing feature of swimming is that individual differences in technique (efficiency) are more important to performance than i the case i cycling (Åstrand 1952 Åstrand

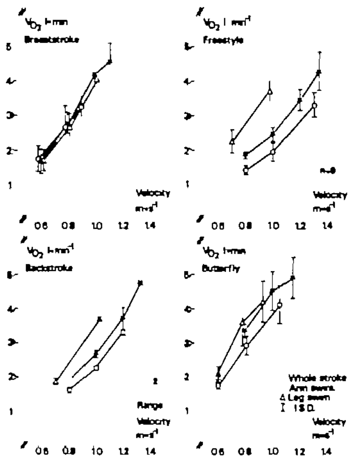


Fig 28 Mean values for oxyge uptake during swimming with arm strokes leg kicks and the stroke as a whol in relatio to velocity (From VII)

.960 Hermansen & Saltin 1969) and running (Margaria et al 1963 Hermansen & Salti 1969) and that efficiency increases markedly with training (VI)

Oxygen ptake in the breaststroke at a give submaximal speed was the same irr spective of wh the the swimme sed th ntire swimming style or arm strokes only or leg kicks (Fig 28) From th propulsio point of view arm strokes were most importa t in the other three swimming styles whereas leg kicks especially in the freestyle were le s economical (Fig 28) It should be noted in this respect that during swimming with arm trokes alone the body probably attained a more favourable position in the wat with cork plate between the swimmer s thighs than without s pport (Fig 5 p 19) However the body position should be similar to the one in swimming with th nt re stroke when leg action exerts a lift on the lower part of the body Th highest o ygen ptake which co ld be produced with

arm strokes only or leg kicks only amounted to 71 83% and 79 90% respectiv ly of highest oxygen uptake for the entire swimming style Maximal swimming speed with arm strokes alone was l ss than with the entire swimming style owing to the clearly lowe oxygen uptake but only slightly lower in the freestyle due to the high efficiency of arm strokes here (VI)

Thus there were variations in efficiency both among the diffe ent swimming styles and even within each swimming style with respect to a m stroke and leg kick efficiency It is therefore important for the swimmer to give priority to the movements which provide the greatest propulsive efficiency especially when energy supply may be a limiting factor In this context it sho ld be kept n mind that the highest energy output which can be produced with the different types of swimming strokes does vary and th efore may have an effect on endurance in the different swimming styles

Concluding remarks

The load on the skeleton joints and ligaments is slight in water owing to the buoyancy To most people treading wate and swimming at a modest speed impose a load on the oxygen transport organs whose magnitude s equal to or g eater than in fast walking These ci cumstances make swimming a suitable form of physical training especially in previously untrained sedentary and olde ubjects In th se categories of people training by running sometime implicat s troublesome complications from joints tendons and muscles of th lowe ext emities (Kilbom et al 1989) Some caution may be advised for people with heart trouble s sw m ming leads to a greater rise in blood pressure than does running

One practical consequence of the very large variations in nergy metaboli m noted in different people swimming at a given speed and using different sw mm ng style is worthy of note A top swimmer swam the freestyle at a speed f 0 6 m s ^{1} with an energy output of 560 W whe ea another well-t a ned pe so (u trained swimmer) needed all of 1100 W (Fig 8) Total energy consumption ov a giv n period of time may vary strikingly depending on the wimming styl sed (F g 25) The popular statem t that swimming for 10 min consum s 400 kJ an over simplif cat on a is lso the case in comparison of the nergy yi lded by various work form

A few conclus on of pract cal significance should be furthe nd lined Max mal oxyg n uptak during running can not be employed to predict performance i w m ming It i obviou that a relatively large change in oxygen uptak may tak pla during maximal swimming without affecting maximal oxygen uptake during running and that inte sive swim training need not be linked to any increa e in maximal oxyg n ptake during running

When heart rate is used as an indicator of the load on oxygen transporting organs during training it should be remembered that this variable is clearly lower in maximal swimming than in maximal running and cycling

At Swedish latitudes in which outdoor water temperatures seldom exceed 20°C a long stay in cold water presents an obvious risk which is especially pronounced in thin people and small children The great heat conductance of water may produce a drop in body temperature of several degrees within half an hour This risk factor must therefore be kept in mind and measures taken to counteract the cooling of the body during activities in cold water

SUMMARY

1 Man s physiological responses to swimming were studied in 87 subjects of varying skill and training status in swimming A swimming fl me provided cellent facilities for more detailed analyses of circulatory and resp rat ry responses as well as energy exchanges of swimming man during more controlled conditions tha was possible in previous investigations Running and ycl ng tests were also performed for purposes of comparison

2 Th main loading factor during wat r treading and swimming at low speed was the body s sinking force (W_F) (1 6 4 7 kp for female and 4 9 5 8 kp fo male subjects) Oxygen uptake during water treading was given by the equat on $\dot{V}O_2$ = 0 33 x W_F (r = 0 96) The sinking force displayed a strong correlati with different anthropometric variables e g fat free soft tissue (r = 0 90) and fat weight (r = 0 76)

3 At moderate and higher swimming peeds the drag on the body increa s exponentially and constitutes the main loading factor Oxygen ptake during swimming at a given submaximal speed was dependent on degree of swimming training body dimensions swimming technique and swimming style Th s oxyge uptake at a given submaximal speed wa generally higher for ntrained subjects and tall subjects compared with trained swimmers and small subjects respectively The increase in oxygen uptake with increasing swimming speed wa linear or slightly exponential Maximal oxyg n uptake in swimming wa for lite swimmers 6–7% lower tha in running and approximately th same in cycling Corresponding value for untrained swimmers were 20% and 10% lower respectively

4 Whe the body was submerged vital capacity wa 10% red ced and the expiratory reserve volum was 1 ss than 1 l as compared with 2 5 l air The increase i tidal volume was effected in water excl sively by the s of th inspiratory reserve volume At corre ponding submaximal $\dot{V}O_2$ level $\dot{V}_E$ and V_E VO_2 were pproximately th same in swimming and running During maximal swimming mean values for V_E and V_E VO_2 were significantly lower than i maximal running o cycling Alveolar ventilation per breath wa greater during both submaximal and maximal swimming as compared with running Value for arterial O_2 satur ation O_2 and CO_2 content and O_2 and CO_2 pressure during both ubmaximal and maximal work were th same i swimming and running

5 Heart rat cardia output and stroke volum during submaximal swimming were of the same magnitude and increased with increasing speed in approximat ly the same way as during running Heart rate wa significantly lowe (12 15 beats

min^{-1}) in maximal swimming than in maximal running Stroke volume was the same while cardiac output was lower in maximal swimming compared with maximal running Maximal $a-\bar{v}$ O_2 difference in swimming was 14 8 vol% and in running 16 0 vol% In exercising leg the a-v O_2 difference was 17 5 vol% and 17 7 vol% in maximal swimming and running respectively

6 In cold water (18°C) thin subjects suffered a 0 2-1 6°C fall in T_{es} in conjunction with 20 min of submaximal swimming In water at 18°C and 26°C changes in T_{es} were related to individual values for skinfold thickness The convective heat transfer coefficient for the heat flow from skin to water was in still water 230 W m ^{2} °C ^{1} and in flowing water 460 W m ^{2} °C ^{1} The value for the coefficient during swimming was 580 W m ^{2} °C^{-1} and it was independent of swimming speed T_{sk} T_w was 1-2°C at rest in still as well as in flowing water and always less than 1°C during swimming T_m T_{es} was always positive the difference being smallest in cold water (18°C) Mean skin conductance during swimming in water at 18°C was relatively independent of speed and approximately 20 W m ^{2} °C ^{1} while in water at 34°C mean skin conductance increased exponentially with T_{es} and averaged 100 W m ^{2} °C ^{1} at 0 75 m s ^{1} and T_{es} = 37 8°C

7 *With lowered internal temperatures (T_{es} < 37°C) shivering occurred in subjects* both at rest and during swimming Thus oxygen uptake was elevated by approximately 0 5 l min ^{1} at a given swimming speed In maximal swimming oxygen ptake was 6-18% educed for subjects with T_{es} < 37°C than at normal or raised T_{es} It was concluded that the elevated oxygen uptake in submaximal swimming pl s the reduced maximal oxygen uptake severely restrict a swimmer s performance and red ce his endurance

8 Breaststrok and butterfly swimming demanded 1 2 l min ^{1} higher oxygen uptake at a g v n ubmaximal speed than did freestyle and backstroke Mechanical efficiency in swimming was low 4 6% in breaststroke and 6-7% in free style by el te swimmer Wh n using only the arms in freestyle swimming efficiency was 7 7% while the leg kicks displyed poor efficiency (2-4% in breaststrok nd 1 % in freestyle)

9 Maximal oxygen uptake for mal elite swimmers was 5 1 l min ^{1} in swimming and 5 4 l min ^{1} in running Corr sponding values for female swimmers were 3 4 and 3 6 l mi ^{1} I relation to body weight maximal oxygen uptake in running amounted to 69 and 5 ml m n ^{1} kg ^{1} for male and f male swimmers respectively Maximal oxygen pt k in sw mming varied as a consequ nce of swimming training while during the same period of time maximal oxygen uptake in running remained relatively unchanged In other words maximal oxygen uptake in running

is not representative for performance in swimming and training of a swimmer s maximal oxygen uptake should to the greatest possible extent take place by swimming

ACKNOWLEDGEMENTS

This work was performed at the Department of Physiology Gymnastik och idrotts högskolan Stockholm Economic and laboratory facilities were also provided by the Work Physiology Division National Board of Occupational Safety and Health Stockholm I am greatly indebted to Stenberg-Flygt AB Solna for providing the swimming flume and excellent service in running it

I wish to express my deep gratitude to Professor Per-Olof Åstrand for his guidance and great interest in my work During fruitful discussions I received advice and ideas of vital importance for my work

I am greatly indebted to Professor Irma Åstrand for unfailing support and valu able advice to Professor Erik Hohwü Christensen Professor Bengt Saltin Associ ate Professor Björn Ekblom and Gymn Dir Ulf Bergh for helpful discussions on my work

I wish to thank my colleagues for pleasant and inspiring collaboration

My sincere thanks also to the entire staff of the respective laboratories for untiring efforts and excellent assistance during the course of the investigation

To all other colleagues and friends who in one way or another contributed to the completion of this thesis I express my warmest gratitude

The studies were supported by research grants from the Swedish National Associ ation against Heart and Chest Diseases and the Research Council of the Swedish Sports Federation

REFERENCES

ADRIAN M J M SINGH and P V KARPOVICH Energy cost of leg kick arm stroke and whole crawl stroke J Appl Physiol 21 1765 1766 1966

AGOSTINO E G GURTNER G TORRI and H RAHN Respiratory mechanics during submersion and negative-pressure breathing J Appl Physiol 21 251 258 1966

ALLEY L E An analysis of water resistance and propulsion in swimming the crawl stroke Res Quart 23 253-270 1952

ANDERSEN H T Physiological adaptations in diving vertebrates Physiol Rev 46 212 243 1966

ANDERSEN K L Energy cost of swimming Acta chir scand Suppl 253:169-174 1960

ARBORELIUS M JR U I BALLDIN B LILJA, and C E G LUNDGREN Hemodynamic changes in man during immersion with the head above water Aerospace Med 43:592 598 1972

ARISTOTLE Problems: Books I XXXVIII English translation by W S Hett London H ieneman 1936

ARMITAGE P Statistical methods in medical research Oxford and Edinburgh Blackwell 1971

ASMUSSEN E and N -G KRISTIANSSON The diving bradycardia in exercising man Acta physiol scand 73:527 535 1968

ÅSTRAND I Aerobic work capacity in men and women Acta physiol scand 49 Suppl 169 1960

ÅSTRAND P -O Experimental Studies of Physical Working Capacity in Relation to Sex and Age Copenhag n Ejnar Munksgaard 1952

ÅSTRAND P -O and S ENGLESSON A swimming flume J Appl Physiol 33: 514 1972

ÅSTRAND P O L ENGSTRÖM B O ERIKSSON P KARLBERG I NYLANDER B SALTIN and C THORÉN Girl swimmer Acta paediat Suppl 147 1963

ÅSTRAND P -O and K RODAHL Textbook of work phy iology New York McGraw 1970

ÅSTRAND P -O and B SALTIN Oxygen uptake during the first minutes of heavy muscular exercise J Appl Physiol 16:971 976 1961 a

ÅSTRAND P -O and B SALTIN Maximal oxygen uptake and heart rate in various types of muscular activity J Appl Physiol 16 977 981 1961 b

BERGMAN S A JR J F CAMPBELL, and K WILDENTHAL Diving reflex in man, its relation to isometric and dynamic exercise J Appl Physiol 33 27 31 1972

BEVEGÅRD S A HOLMGREN and B JONSSON Circulatory studies in well trained athletes at rest and during heavy exercise with special reference to stroke volume and the influence of body position Acta physiol scand 57 26 50 1963

BRICK I Circulatory responses to immersing the face in water J Appl Physiol 21 33 36 1966

BULLARD R W and G M RAPP Problems of body heat loss in water immersion Aerosp Med 41 1269 1277 1970

BURTON A C and H C BAZETT A study of the average temperature of the tissues of the exchang s of heat and vasomotor responses in man by means of a bath calorimeter Amer J Physiol 117 36-54 1936

BURTON A C and O G EDHOLM Man in a cold environment London Edward Arnold 1955

CARLSON L D A C HSIEH F FULLINGTON, and R W ELSNER Immersion in cold wate and body tissue insulation J Aviation Med 29,145-152 1958

CHRISTENSEN E H R HEDMAN and I HOLMDAHL The influence of rest pa ıses on mechanical efficiency Acta physiol scand 48 443-447 1960

COUNSILMAN J E Forces ı swimming two types of crawl stroke Res Quart 26 127 139 1955

COUNSILMAN J E The Scie ce of Swimming Englewood Cliffs Prentice Hall Inc 1968

CRAIG A B JR nd M DVORAK Thermal regulation of man exercising during water ımmers on J Appl Physiol 25 28 35 1968

DEMEDTS M and N R ANTHONISEN Effects of increased external airway resistance dur ng steady stat exerc se J Appl Physiol 35 361 366 1973

DEPOANNE R F PIRI AY J DUJARDIN, and J M PETIT Mechanical properties of the lungs during swimmin J I Ist I t Symposi m on Biomechanics in Swimming Ed L Lewillie and J P Clarys Bruss ls Université libre de Bruxelles Laboratoire de l'Effort 1971 pp 207 215

DIXON R W JR and J A FAULKNER Cardiac outputs dur ng m m
running and swimming J Appl Physiol 30 653 656 19

DUBOIS-REYMOND R Zur Physiologie des Schwimmens Arch f
Physiol 29 252 279 1905

DÖBELN W VON Human standard and maximal metabolic t
fatfree body mass Acta physiol scand Suppl 1 6 1956

DÖBELN W VON Anthropometric determination of fat fre bod
med scand 165 37 40 1959

DÖBELN W VON and B LINDELL Some aspects of radon co t n t k w
ing ingestion Arkiv f Fysik 27:531 572 1964

EDWARDS D A W Observations on the distribution of subc tu
Sci 9 259 270 1950

EKBLOM B Effect of physical training on oxygen transport sy t m A t
physiol scand Suppl 328 1969

EKBLOM B and L HERMANSEN Cardiac output in athlet s J Appl Ph ol
25 619 625 1968

ERIKSSON B O Physical training oxygen supply and m s l metabol m
11 13-year old boys Acta physiol scand Suppl 384 1972

FLOOK V and G R KELMAN Submaximal exe cise with increased p ratory
resistance to breathing J Appl Physiol 35 379 384 1973

FOLKOW B U HAGLUND M JODAL and O LUNDGREN Blood flow n the
calf muscle of man during heavy rhythmic exe cise. Acta phys ol scand 81
157 163 1971

FREDERIKSEN R Om svømningens fysiologi 2 T Læg mæ Ise 16 49 54
1945

GLOSSARY COMMITTEE of the IUPS Glossary on espirat o and ga xchang
J Appl Physiol 34 549 558 1973 a

GLOSSARY COMMITTEE of the IUPS Glo sary of term for thermal phy iology
J Appl Physiol 35 941 961 1973 b

GOLDEN F ST C and G R HERVEY A cla s experiment o imm io hypo
thermia J Phy iol Lond 227 35P 36P 1972

GOLLNICK P D R B ARMSTRONG B SALTIN C W SAUBERT IV W L
SEMBROWICH and R E SHEPHERD Effect of training on enzyme act vity nd
fibe compo ition of human keletal muscle J Appl Phy iol 34 107 111
1973

GOLLNICK P D R B ARMSTRONG C W SAUBERT IV K PIEHL and B SALTIN Enzyme activity and fiber composition in skeletal muscle of untrained and trained men J Appl Physiol 33 312 319 1972

GOODWIN A B and G R CUMMING Radio telemetry of the electrocardiogram fitness tests and oxygen uptake of water-polo players Canad Med Ass J 95 402 406 1966

HANSEN E Om bestemmelse af vandets modstand under svømning T Legemsøvelser 20 36-45 1955

HEMMINGSEN I Energiomsaetningen under svømning hos maend og kvinder T Legemsøvelser 22 53-62 1957

HERMANSEN L and W VON DÖBELN Body fat and skinfold measurements J clin Lab Invest 27 315 319 1971

HERMANSEN L and B SALTIN Oxygen uptake during maximal treadmill and bicycle exercise J Appl Physiol 26 31 37 1969

HOLMÉR I Energiomsattning under simning T Gymnastik 10:421-432 1973

HONG S K P CERRETELLI J C CRUZ and H RAHN Mechanics of respir ation during submersion in water J Appl Physiol 27 535 538 1969

KARPOVICH P V Water resistance in swimming Res Quart 4 21 28 1933

KARPOVICH P V and N MILLMAN Energy expenditure in swimming Amer J Physiol 142 140-144 1944

KEATINGE W R The effects of subcutaneou fat and of previous exposure to cold on the body temperature peripheral blood flow and metabolic rate of men in cold wat r J Physiol 153 166-178 1960

KEATINGE W R The effect of work and clothing on the maintenance of the body temperat re in water Quart J Exp Physiol 46 69 82 1961

KEATINGE W R Survival in cold water Oxford & Edinburgh Blackwell 1969

KENT M R and J ATHA Selected critical transient body positions in breast stroke and their influence upon wat resistance In 1st International Sympo sium on Bi mechanics Swimming Eds L Lowill e nd J P Clarys Brussels Université libre de Bruxelles Laboratoire de l'Effort 1971 pp 119 125

KILBOM Å Physical training wome Scand J clin Lab Invest 28 S ppl 119 1971

KILBOM Å L H HARTLEY B SALTIN J BJURE G GRIMBY and I ÅSTRAND Physical training in sedentary middle-aged and older men Scand J clin Lab Invest 24 315 322 1969

KLISSOURAS V Energy metabolism in swimming the dolphin stroke Int Z angew Physiol 25: 142 150 1968

KREBS H In: Oxygen in the Animal Organism Eds F Dickens E Neil and W F Widdas New York Pergamon Press 1964 p 304

LEFÈVRE M J Recherches sur la conductabilité de la peau de l'organisme vivant et sur ses variations en fonction de la temperature extérieure Étude sur l'homme J de Physique 10:380 388 1901

LILJESTRAND G and J LINDHARD Über das Minutenvolumen des Herzens beim Schwimmen Skand Arch Physiol 39 64 77 1919

LILJESTRAND G and N STENSTRÖM Studien über die Physiologie des Schwimmen Skand Arch Physiol 39 1 63 1919

MAGEL J R and J A FAULKNER Maximum oxygen uptakes of college swimmers J Appl Physiol 22 929 938 1967

MCARDLE W D R M GLASER and J R MAGEL Metabolic and cardiorespiratory response during free swimming and treadmill walking J Appl Physiol 30 733 738 1971

MARGARIA R P CERRETELLI P AGHEMO and G SASSI Energy cost of running J Appl Physiol 18 367–370 1963

MOORE T O Y C LIN D A LALLY and S K HONG Effects of temperature immersion and ambient pressure on human apneic bradycardia J Appl Physiol 33 36–41 1972

MORGAN T E L A COBB F A SHORT R ROSS and D R GUNN Effect of long-term exercise on human muscle mitochondria In Muscle Metabolism During Exercise Ed B Pernow and B Saltin New York Plenum 1971 pp 87–95

NIELSEN B Thermoregulation in rest and exercise. Acta physiol scand Suppl 323 1969

PATTENGALE P K and J O HOLLOSZY Augmentation of skeletal muscle myoglobin by a program of treadmill running Amer J Physiol 213:783 785 1967

PAULEV P Cardiac dysrhythm during breath holding and water immersion in man Acta physiol scand 73:139 150 1968

DI PRAMPERO P E D R PENDERGAST D W WILSON and D W RENNIE Energetics of swimming in man J Appl Physiol 1974 (In press)

PUGH L G C and O G EDHOLM The physiology of channel swimmers Lancet II 761 768 1955

RAPP G M Convection coefficients of man in a forensic area of thermal physiology- Heat transfer in underwater exercise J Physiol Paris 63 392 396 1971

RENNIE D W P DI PRAMPERO and P CERRETELLI *Effects of water immersion on cardiac output heart rate and stroke volume of man at rest and during exercise* Medicina dello Sport 24 223 228 1971

ROWELL L B J A MURRAY G L BRENGELMANN and K K KRANING II Human cardiovascular adjustments to rapid changes in skin temperature during exercise Circ Res 24 711-724 1969

SALTIN B Oxygen transport by the circulatory system during exercise in man In: Limiting Factors of Physical Performance Ed J Keul Stuttgart Georg Thi me Publ 1973 pp 235 252

SALTIN and P -O ÅSTRAND Maximal oxygen uptake in athletes J Appl Physiol 23:353 358 1967

SALTIN B A P GAGGE U BERGH and J A J STOLWIJK Body temperatures and sweating during exhaustive exercise J Appl Physiol 32 635 643 1972

SCHOLANDER P F H T HAMMEL H LEMESSURIER E HEMMINGSEN and W Garey Circulatory adjustment in pearl divers J Appl Physiol 17 184 190 1962

SLOAN R E G and W R KEATINGE Cooling rates of young people swimming in cold water J Appl Physiol 35 371 375 1973

STENBERG J P -O ÅSTRAND B EKBLOM J ROYCE and B SALTIN Hemodynamic response to work with different muscle groups sitting and supine J Appl Physiol 22 61 70 1967

STRAUSS M B Physiological aspects of mammalian breath hold diving: a review Aerospace Med 41:1362 1381 1970

STRÖM G The influ nce of anoxia o lactate utilization in man after prolonged muscular work Acta physiol scand 17 440-451 1949

STRØMME S B D KEREM and R ELSNER Diving bradycardia during rest and exercise and its relation to physical fitness J Appl Physiol 28 614 621 1970

WERKÖ L The infl ence of positive pre sure breathing on the circulation in man Stockholm Bonniers 1947

WHIPPLE G H The hemoglobin of striated muscle 1 Variation due to age and exercise Amer J Physiol 76 693-707 1926

WITHERSPOON J M R F GOLDMAN and J R BRECKENRIDGE Heat transfer coefficients of humans in cold water J Physiol Paris 63 459-462 1971

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 408

ADAPTIVE CHANGES OF CARDIOVASCULAR DESIGN IN SPONTANEOUS AND RENAL HYPERTENSION

Hemodynamic studies in rats.

BY

YEN LUNDGREN

GÖTEBORG 1974

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 408

ADAPTIVE CHANGES OF CARDIOVASCULAR SPONTANEOUS AND RENAL HYPERTENSION

Hemodynamic studies in rats

BY

YEN LUNDGREN

GÖTEBORG

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 408

From the Departement of Physiology
University of Göteborg, Sweden

ADAPTIVE CHANGES OF CARDIOVASCULAR DESIGN IN SPONTANEOUS AND RENAL HYPERTENSION

Hemodynamic studies in rats.

BY

YEN LUNDGREN

GÖTEBORG 1974

This summary is based on studies reported in the following papers:

I Structurally based increase of flow resistance in spontaneously hypertensive rats
B Folkow M Hallbäck Y Lundgren and L Weiss Acta physiol scand 1970 79 373-378

II Renal vascular resistance in spontaneously hypertensive rats
B Folkow M Hallbäck Y Lundgren and L Weiss Acta physiol scand 1971 83 96-105

III Rate and extent of adaptive cardiovascular changes in rats during experimental renal hypertension
Y Lundgren M Hallbäck L Weiss and B Folkow Acta physiol scand 1974 In press

IV Regression of structural cardiovascular changes after reversal of experimental renal hypertension in rats
Y Lundgren Acta physiol scand 1974 In press

V Blood pressure and vascular design in renal hypertension in rats after prolonged propranolol treatment
Y Lundgren Acta physiol scand 1974 In press

The papers are referred to in the text by their Roman numerals

Printed [illegible] 74 [illegible]

CONTENTS

INTRODUCTION 5

PREVIOUS STUDIES 6

History 6

Background of increased arterial pressure 7
Cardiac output 7
Peripheral resistance 8

HEMODYNAMIC CONSEQUENCES OF MORPHOLOGICAL VASCULAR CHANGES 12

Theoretical considerations 12

Earlier hemodynamic studies of the resistance vessels 13

AIM OF THE PRESENT STUDY 18

METHODOLOGICAL PRINCIPLES 20

The experimental animal models 20
Spontaneously hypertensive rats (SHR) 20
Renal hypertensive rats (RHR) 21

Blood pressure and heart rate measurements 21

General perfusion procedures 22
Perfusion medium 22
Pressure-flow relationships during maximal dilatation 23
Noradrenaline (NA) infusion during constant flow conditions 24

Measurements of the water conte t of the aortic wall 26

Statistical evaluations 27

RESULTS AND COMMENTS 28

Structural changes of the total systemic vascular bed i SHR (paper I) 28

Structural changes of the SHR renal vascular bed (paper II) 29

Rate and extent of development of adaptive cardiovascular changes in RHR (paper III) 32
Regression of hypertensive cardiovascular changes upon reversal of renal hypertension in rats (paper IV) 36
Propranolol treatment in RHR (paper V) 40
GENERAL DISCUSSION 42
Primary hypertension 44
Renal hypertension 45
Regression of cardiovascular changes 47
SUMMARY OF THE PRESENT RESULTS 48
ACKNOWLEDGEMENTS 50
REFERENCES 51

INTRODUCTION

Increased activity of the precapillary smooth muscles has for decades been considered to be the background of the increased resistance to flow in established hypertension Very little attention has been paid to the possible hemodynamic consequences of the media hypertrophy in arteries and precapillary resistance vessels despite the fact that such changes in vascular design have been recognized by morphologists for over 100 years Recently quantitative hemodynamic analyses Initially in man (see Folkow 1956 Sivertsson 1970) and later in spontaneously hypertensive rats (e g Folkow et al 1970 1973) have shown that structural changes of the resistance vessels are indeed of great hemodynamic importance Thus the mentioned studies revealed that the resistance vessels display an increased resistance even during maximal dilatation and exaggerated resistance increases upon smooth muscle contractions implying that esistance will be increased even when smooth muscle tone is normal This effect of a changed vascular design may be denoted structural autoregulation as a structural correlate to the functional autoregulation characterizing precapillary vessel responses upon acute changes in pressure

The present series of investigations based on five individual studies that are below denoted I – V was performed in orde to add further knowledge to the background of the incr ased resistance to flow both in primary hypertension and for comparison in the most common type of secondary hypertension i e renal hypertension For such purposes the adaptive structural changes in cardiovascular design we e studied in more detail in the spontaneously hypertensive rat (SHR; Okamoto 1969) which is probably the best animal model so far for essential hypertension in man and in the renal hypertensive at (RHR) In SHR studies utilizing quantitative perfusion analyses were performed con cerning esistance vessel design of the entire systemic vascular bed and of the renal vascular circuit Furthermore the extent and time course of the development and egression of cardiovascular hypertrophy we e studied utilizing the enal hypertensive at Finally since preventive propranolol treatment seems to exert a protective influence in SHR against the progress of hypertension and hypert ophic vascular changes (Folkow Lundgren and Weiss 1972) it was explored how the same type of preventive treatment with β-receptor antagonists affected the development of enal hypertension and the associated hypertensive vascular changes

PREVIOUS STUDIES

History

Hypertensive disease was known to exist for a long time before the technique of indirect pressure measurement was developed during the latter half of the nineteenth century Therefore characterization of the disease was initially based mainly on the morphological changes of heart arteries and kidneys after death Bright (1836) described a se les of cases with albuminuria in combination with three different stages of degenerative kidney and found a marked hypertrophy of the left ventricle In a large number of these cases he did not find any organic cause for the cardiac hypertrophy and suggested that an altered quality of the blood might either have affected the heart directly or that it so affected the vessels that an enhanced cardiac force was needed

Later Gull and Sutton (1872) concluded that the arterioles and the capillaries were altered throughout the body in chronic Bright s disease due to a hyalin fibroid formation in the arteriolar walls and a hyalin granular change in the capillaries These morphological changes in the arterioles and capillaries were thought to be the primary and causative change in chronic Bright s disease with contracted kidney However Ewald (1877) confirming Johnson s (1868) findings that the media hypertrophy was associated with cardiac hypertrophy considered the vascular changes to be a consequence rather than the cause of hypertension

Mahomed (1879 1881) who studied chronic B ight s disease found that the cardiovascular changes often preceded the renal changes and concluded: "What has been cause in one case may be the result in thus general disorder may cause high arterial pressure and this in its turn kidney changes while on the other hand kidney changes may be primary and acute and they may in their turn produce impurity of blood and this general high pressure But whether we read the tale backwards or forwards it is the same tale in the end

A hypertensive disorder with quite a different course than Bright s disease of the kidneys was first recognized by Huchard (1889) von Basch (1893) and Allbutt (1915) The term essential hyper tension was first used by Frank (1911) and the modern classification of hypertension is based on a combined clinical and pathological study by Volhart and Fahr (1914) However since the etiology of essential hypertension has emained unknown the question as to whether genetical factors were i volved arose early In 1923 Weitz noted a more frequent occurrance of high blood pressure in brothers and sisters of

patients with essential hypertension than in control subjects

The earlier discovered media hypertrophy was confirmed by Turnbull (1915) who stated that it was constantly associated with high blood pressure and well correlated to the degree of the cardiac hypertrophy Furthermore Kernohan Anderson and Keith (1929) found that media hypertrophy was the major component of the increased thickness of the arterial wall relative to lumen

To study hypertensive disease in more detail different kinds of experimental hypertension were introduced The most common methods used were renal artery constriction (Goldblatt et al 1934 Drury 1938 Pickering and Prinzmetal 1938) and later the administration of deoxycorticosteroneacetate (DOCA) (e g Selye Hall and Rowley 1943) Generalized media hypertrophy in medium sized and small arteries was observed in dogs with experimental renal hypertension (Goldblatt 1938 and Child 1938) The recent introduction of animal models with primary hypertension which more closely resemble the most important type of hypertension in man essential hypertension provides a valuable experimental tool in the study of hypertensive disease

Background of increased arterial pressure

According to Poiseuille s law and Frank s formula mean arterial pressure is determined by the relationship between cardiac output and peripheral resistance Thus an increased arterial pressure may be due to an increased cardiac output and/or to an increased peripheral resistance An increased peripheral resistance may in turn be due to a raised viscosity of the blood an increased length of the vessels and/or to a reduced cross-sectional area of the vascular bed

Cardiac output

An increased cardiac output has been observed in early phases of essential hypertension by e g Werkö and Lagerlöf (1949) Sannerstedt (1966) Lund-Johansen (1967) Julius and Conway (1968) Frohlich et al (1970) and Julius and Schork (1971) However in established essential hypertension cardiac output usually seems to be withi normal limits during rest (e g Goldring and Chasis 1944 Freis 1960 Bello Sevy and Harakal 1965 Frohlich et al 1970) In renal hypertension a transient early increase in cardiac output has often been noted (g Ledingham and Cohen 1964 Ferrario Page and McCubbin 1970 Bianchi et al 1972) but also in this type of hypertension the increased resistance seems to dominate later phases Thus in both primary and

secondary (renal) hypertension the increased resistance is the dominating feature in the established phase

Peripheral resistance

According to Poiseuille s law resistance to flow is determined by viscosity vascular length and internal radius Blood viscosity seems to be normal in established essential hypertension (Pickering 1968) In young hyper tensive subjects blood viscosity as measured in vitro was found by Tibblin et al (1966) to be 20 per cent higher than in normotensives Only slight changes in hematocrit and plasma viscosity were noted However in vitro measurements of blood viscosity must be judged with caution since they are often unreliable indicators of the viscosity in vivo (Djojosugito et al 1970) In renal hypertension changes in hematocrit have not been found (e g Ledingham and Pelling 1967 Ferrario Page and McCubbin 1970) No studies provide evidence that changes in the length of resistance vessels are of such magnitude to be of any relevance for the increased resistance to flow It is therefore generally agreed that the increased resistance in hypertension is primarily due to a reduction in average internal radius of the resistance vessels and it has as mentioned above been more or less generally assumed that this should be due to an increased vascular smooth muscle activity Such an increased smooth muscle activity could then in turn be due to a greater sympathetic activity increased amounts of humoral vasoconstrictor agents increased smooth muscle sensitivity or "reactivity to constrictor influences changes in the electrolyte balance and/or to changes in basal myogenic tone

Sympathetic tone: There is no clear evidence of any constantly increased sympathetic tone at rest either in established primary hypertension (see Pickering 1968 Wallin Delius and Hagbarth 1973) or in secondary renal hypertension (cf Page and McCubbin 1968) This by no means denies that sympathetic influences are of great importance but act in a different manner as will be discussed later

Humoral constrictor agents. Increased amounts of circulating constrictor agents have not been found in primary hypertension at least not in concentrations high enough as to alone explain the increased resistance level (cf Pickering 1968 Okamoto 1972) The importance of the renin-angiotensin system and its interaction with the sympathetic nervous system particularly in renal hypertension has been much debated but is still not exactly understood (cf Page and McCubbin 1968 Joy 1971)

Vascular reactivity: Concerning increased smooth muscle sensitivity or

"reactivity to constrictor agents this possibility has been extensively studied in a great number of studies of blood pressure changes and changes in total or regional resistance to constrictor agents (cf Freis 1960 Page and McCubbin 1965 Doyle 1968 Laverty McGregor and McQueen 1968 Page and McCubbin 1968 Pickering 1968 Sivertsson 1970 Mendlowitz 1973) However the results are often contradictory and sometimes even confusing since both experimental approach and definitions differ considerably It should be realized that increased blood pressure responses and I creased vascular responses to injected constrictor agents can have widely different backgrounds and that furthermore increased vascular reactivity does not necessitate any increased smooth muscle reactivity or sensitivity In case structural increases in wall/lumen ratio are also present (see also Sivertsson 1970 Tobian 1972 Johansson 1974)

Electrolytes: It has been suggested that changes in electrolyte composition might lead to increased smooth muscle activity (Friedman Friedman and Nakashima 1957 Pickering 1968) According to Freis (1960) there is no evidence of any decisive changes in electrolyte composition leading to increased vascular smooth muscle tone in established uncomplicated primary hypertension However changes in the ioncomposition have been found in spontaneously hypertensive rats (Nagaoka Kikuchi and Aramaki 1970 Jones 1973) but further studies are needed to settle this question Consideri g enal hypertension alterations in renin-angiotensin-aldosterone secretions may influence the lectrolyte balance in such a way as to increase smooth muscle tone but such mechanisms have not so far been shown to be of major importance (cf Page and McCubbin 1968)

Basal myogenic tone: Hardly any studies are available that more directly deal with the possibility of an increased myogenic tone as the background of the raised resistance (cf Freis 1960)

Structural changes in resistance vessel design: The increased flow resistance found in hypertensive subjects may be due to mechanisms quite different in nature from those described above Thus instead of a functional luminal narrowing it is a priori quite possible that a structural luminal reduction exists as a result of an increased wall thickness For exampl Tobian and Binion (1952) (cf Tobian 1972) suggested that an increased amount of water and electrolytes in the arteriolar walls i e water logging might exist causing an increased wall thickness with a conseq ent luminal narrowing This hypothesis was mainly based on analyses of the water and electrolyte contents in conduit arteries from patients who died in hypertensive disease How-

secondary (renal) hypertension the increased resistance is the dominating feature in the established phase

Peripheral resistance

According to Poiseuille s law resistance to flow is determined by viscosity vascular length and internal radius Blood viscosity seems to be normal in established essential hypertension (Pickering 1968) In young hypertensive subjects blood viscosity as measured in vitro was found by Tibblin et al (1966) to be 20 per cent higher than in normotensives Only slight changes in hematocrit and plasma viscosity were noted However in vitro measurements of blood viscosity must be judged with caution since they are often unreliable indicators of the viscosity in vivo (Djojosugito et al 1970) In renal hypertension changes in hematocrit have not been found (e g Ledingham and Pelling 1967 Ferrario Page and McCubbin 1970) No studies provide evidence that changes in the length of resistance vessels are of such magnitude to be of any relevance for the increased resistance to flow It is therefore generally agreed that the increased resistance in hypertension is primarily due to a reduction in average internal radius of the resistance vessels and it has as mentioned above been more or less generally assumed that this should be due to an increased vascular smooth muscle activity Such an increased smooth muscle activity could then in turn be due to a greater sympathetic activity increased amounts of humoral vasoconstrictor agents increased smooth muscle sensitivity or "reactivity to constrictor influences changes in the electrolyte balance and/or to changes in basal myogenic tone

Sympathetic tone: There is no clear evidence of any constantly increased sympathetic tone at rest either in established primary hypertension (see Pickering 1968 Wallin Delius and Hagbarth 1973) or in secondary renal hypertension (cf Page and McCubbin 1968) This by no means denies that sympathetic influences are of great importance but act in a different manner as will be discussed later

Humoral constrictor agents: Increased amounts of circulating constrictor agents have not been found in primary hypertension at least not in concentrations high enough as to alone explain the increased resistance level (cf Pickering 1968 Okamoto 1972) The importance of the renin angiotensin system and its interaction with the sympathetic nervous system particularly in renal hypertension has been much debated but is still not exactly understood (cf Page and McCubbin 1968 Joy 1971)

Vascular reactivity: Concerning increased smooth muscle sensitivity or

has been most convincingly demonstrated with different morphological and histochemical techniques but it is exceedingly difficult with such techniques alone to estimate the hemodynamic consequences which in the final end is the crucial point

HEMODYNAMIC CONSEQUENCES OF MORPHOLOGICAL VASCULAR CHANGES

Theoretical considerations

Flow resistance (R) is commonly used as an index of the average internal radius (r_i) of the resistance vessels in hypertension since factors like vascular length and blood viscosity appear to be largely unchanged, as mentioned above Since R is inversely proportional to the fourth power of r_i any change of r_i becomes greatly amplified when expressed as R The resistance at complete vascular smooth muscle relaxation (R_{min}) would then reflect the structurally determined r_i and in a way be an expression of the resting length of the encircling muscle elements Under standardized conditions R_{min} would thus, in a way imply a structurally defined baseline from which current levels of vascular smooth muscle activity can be judged as the ratio between the prevailing R level and R_{min}

However when a wall thickening becomes involved it will amplify the luminal reduction induced by a given smooth muscle shortening thereby distorting the relationship between R and R_{min} Therefore the entire dynamics of resistance vessels will become markedly changed whenever their structurally determined wall/lumen ratio is altered (cf Folkow et al 1973) Concerning adrenergic excitatory influences e g those exerted by the vasoconstrictor fibres the smooth muscle contraction of the resistance vessels is initiated from the outer muscle sheath since all the neuroeffector functions are placed at its adventitial border (cf Ljung 1970) The thicker the media (and other wall layers placed inside the media), the more pronounced the luminal reduction for any given level of smooth muscle shortening since the wall mass constant Thus a vascular hyperreactivity is created whenever the wall/lumen ratio is increased which does not call for any smooth muscle hyperreactivity or hypersensitivity It also follows that the resistance becomes higher for any given level of smooth muscle tone Furthermore in case the lumen is educed even at complete smooth muscle relaxation the very baseline for vascular responses is raised which will increase resistance even more for any given degree of smooth muscle shortening However even in case R_{min} is the same or even decreased an enhanced wall/lumen ratio will create a vascular hyperreactivity of great importance for resistance control

Such changes in vascular design would thus imply a structural autoregulation of the resistance vessels enabling them to reset the resistance equilibrium and the range of dilatation constriction at a higher level without necessitating any increased vascular smooth

muscle tone Further the thicker walls would make the vessels stiffe and the reduced luminal size would balance off the rise in wall tension that would otherwise occur as a result of the raised pressure

With respect to larger arteries the increased stiffness would help to explain the resetting of the baroreceptors (Aars 1969) and the related hypertrophy of the left ventricle will obviously imply a suitable compensation for the imposed work load on the left heart Thus changes of tl is general nature can be expected to have far-reaching hemodynamic consequences when once established in hypertension

Ea lie hemodynamic studies of the resistance vessels

Hemodynamic studies on regional vascular beds in man (Folkow 1956 Folkow G imby and Thulesius 1958 Conway 1963) suggest that the raised flow resistance in established hypertension may predominantly be due to a structural change of the resistance vessels Thus even during complete vascular smooth muscle relaxation resistance to flow in the forearm was increased almost to the same extent as the resting blood pressure Moreover compa ative hemodynamic studies on the hand vas cular bed in hypertensive and normotensive subjects evealed not only an increased resistance at maximal dilatation but also exaggerated in creases in resistance responses to noradrenaline in the hypertensive sub jects though smooth muscle sensitivity to noradrenaline appeared to be largely unchanged (Sive tsson 1970) Thus the resistance vessels of the hypertensive subjects differed from those of normotensive controls first by exhibiting a raised flow resistance even at complete vascula smooth muscle relaxation second by showing no signs of any enhanced smooth muscle activity in the resting equilibrium since the ratio between esting R and R_{min} was not increased and thi d by dis playing a vascular "hyperreactivity" without any clea evidence of enhanced smooth muscle sensitivity These findings strongly suggest the presence of a structural change of the egional esistance vessels so organized that they exhibit an increased wall thickness in combina tion with a decreased luminal size even at maximal dilatation

To allow more detailed hemodynamic studies of resistance vessel design in primary hypertension the spontaneously hypertensive rat (SHR; Okamoto and Aoki 1963) has been used SHR which seem to be the best animal model so far for essential hypertension in man have been developed from normal Wistar rats by inbreeding of animals with higher than average blood pressures Further these rats appear to have a multigenic background of thei hypertension as seems to be the case also in human essential hypertension (cf Pickering 1968)





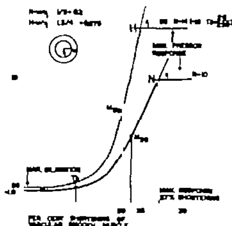


Fig 1 Left part: Average resistance curves for spontaneously hypertensive rats (SHR) and normotensive control rats (NCR) based on the results of 15 paired experiments Right part: Mathematically deduced resistance curves for two hypothetical resistance vessels H and N where H differs from N only in the respect that its media thickness is supposed to be increased 30 per cent encroaching on its lumen even at complete smooth muscle relaxation w/r = ratios of wall thickness to internal radius Note the striking similarities between the relationships of the two sets of resistance curves with respect to: 1 Resistance at maximal dilatation 2 Threshold (Th) dose of noradrenaline 3 Steepness of the curves 4 M_{50} i e 50 per cent of the maximal pressor response to noradrenaline and 5 Maximal pressor response (Folkow et al 1970)

In a series of experiments the isolated hindquarters of one SHR and one matched normotensive control rat (NCR) were perfused in parallel and their hemodynamic characteristics were compared from maximal dilatation up to maximal constriction (Folkow et al 1970) Thus during constant flow conditions noradrenaline (NA) was infused in a stepwise fashion from subthreshold up to supramaximal concentrations

and the resistance responses were recorded giving the mean dose-response curves for SHR and NCR (resistance curves) shown in the left part of Fig 1 When compared to NCR the SHR resistance curve showed the following hemodynamic characteristics:

1 A raised flow resistance at maximal dilatation

2 An unchanged NA threshold (i e the NA concentration producing 25 per cent increase in resistance from the state of maximal dilatation)

3 Exaggerated resistance responses to suprathreshold concentrations of NA expressed as an increased steepness of the resistance curve

4 A shift towards the left of the resistance response that corresponds to 50 per cent of the maximal pressor response to NA

5 A raised maximal pressor response to supramaximal amounts of vasoconstrictor agents i e an increased maximal contractile strength

These 5 characteristics together strongly suggest an increased wall/lumen ratio mainly due to an increased media thickness that also implies a somewhat reduced lumen even at maximal dilatation

However the hemodynamic consequences of such a structural change is best illustrated by exact computations of resistance changes in idealized model vessels as shown in the right part of Fig 1 This Figure illustrates mathematically deduced resistance curves for two hypothetical resistance vessels one normotensive N and one hypertensive H The ratio between wall thickness w and internal radius r_i of the normal resistance vessel N is set at 1:5 at maximal dilatation (Van Citters 1966) The same ratio for the hypertensive vessel H is assumed to be 1 3:4 7 Hence the two vessels differ only in the respect that H displays a 30 per cent increase of media thickness which encroaches upon the lumen even at complete smooth muscle relaxation The wall mass is considered to be constant when N and H constrict which is also in agreement with direct measurements on vessels (Baez 1969) It is further assumed that constriction is initiated from the outermost muscle sheath since most of the α-receptors seem to be located at the adventitial muscle surface (Johansson et al 1970) This would imply that the constriction caused by e g exogenous NA displaces inner tissue layers towards the lumen, which then becomes reduced in an exaggerated way and the more so the stronger smooth muscle contraction and the thicker the wall is to start with Further the NA concentration is given in a log scale on the abscissa of the left part of Fig 1 while the percentage shortening of smooth muscle length is instead used on the abscissa for the

vascular models in the right pa t Since log NA concentration is related to decrease of smooth muscle length by the S formed dose-response curves characterizing e g contracting vascular smooth muscle strips the scales of the two abscissae are made as equal as possible in Fig 1 by utilizing such curves for transformation

When comparing the mathematically deduced resistance curves for the two model resistance vessels the relationship between curve H and curve N is in all essential details closely similar to the experimentally observed relationship between the SHR and NCR resistance curves In fact the parallels between the mathematically deduced curves and the experimental curves are so close as to suggest that the extent of structural change of the SHR resistance vessels may have been of a similar or slightly greater magnitude than assumed to be present in H

Considering other types of structural or functional vascular changes proposed to be involved in hypertension an increased vascular smooth muscle sensitivity is incompatible with all the observed characteristics of the resistance curves Threshold NA concentration would then be decreased and the resistance curve would simply be displaced to the left without any increase in steepness Moreover isolated aortic strips from SHR and NCR show no difference in NA sensitivity (Hallbäck Lundgren and Weiss 1971) As to other mechanisms proposed several of these would have to be combined to fit the experimentally obtained resistance curves and in addition any hemodynamic influence of the morphologically well documented media hypertrophy would then have to be disregarded Thus a thickening of only non-contractile wall elements or a mere water logging (Tobian and Binion 1952 Tobian 1972) could explain most differences but not the considerably increased maximal contractile strength of the SHR resistance vessels beyond the minor gain that would result from the smaller r_i according to Laplace s law Moreover a rarification of the resistance vessels a reduction of their size at an unchanged wall/lumen ratio or an increased length could explain the increased resistance at *maximal dilatation and the unchanged NA threshold but not the other* hemodynamic characteristics of the SHR vessels In other words only the presence of an increased media thickness in association with a slightly reduced lumen of the resistance vessels even at maximal dilatation can alone explain all the demonstrated hemodynamic characteristics of the SHR hindquarter vascular bed Thus these results from the so far best animal model of essential hypertension are in complete agreement with the findings in man and with the view that the increased vascular reactivity and flow resistance in established primary hypertension is mainly or entirely caused by the mentioned type of

changed structural design i e an increased media thickness of the resistance vessels

These results are further supported by the finding that the SHR resistance vessels are also proportionally less distensible than the NCR ones both at maximal dilatation and at different levels of smooth muscle tone (Hallbäck Lundgren and Weiss 1974) In addition recent more detailed analyses of the site and extent of the increased resistance in hypertension show that the hypertrophic vascula changes in SHR are solely confined to the precapillary resistance vessels and closely proportional to the increased blood pressure level while the capillary section is largely unchanged and the postcapillary resistance section if anything slightly wider and somewhat less reactive to NA in SHR (Folkow et al 1974)

AIM OF THE PRESENT STUDY

The present study was performed in order to investigate more in detail certain aspects of the cardiovascular structural changes outlined above using the spontaneously hypertensive rat (SHR) and the renal hypertensive rat (RHR) as models Since all similar studies in man were performed on a few regional vascular beds the total systemic vascular bed of SHR was used in the first study (I) to explore whether the previously mentioned structural vascular changes were generalized throughout the systemic circulation and if so whether they were pronounced enough to contribute markedly to the elevated pressure in established primary hypertension

The kidneys are of particular interest in hypertension and an increased renal resistance to blood flow has been almost uniformly reported in essential hypertension (cf Pickering 1968) though it is not settled to what an extent this is due to structural or functional factors For such reasons SHR and NCR kidneys were perfused in parallel to find out whether the renal vascular bed displays any structural narrowing in SHR which would then imply a potential Goldblatt mechanism of possible etiological importance (paper II)

Furthermore it was considered of great importance to find out whether the structural cardiovascular adaptation to increased arterial pressure develops rapidly or if it is a fairly late complication Thus in order to study the extent and exact time course of the mentioned structural changes renal hypertensive rats were used because the onset of this type of hypertension is far more abrupt than in SHR (paper III) Another important question arising is if animals with primary hypertension (SHR) are genetically more prone to develop cardiovascular hypertrophy in response to increased pressure than those with secondary hypertension (RHR) Even a modest quantitative difference in this respect may have considerable hemodynamic consequences for the resistance vessels and their function because of the tendency of functional and structural factors to reinforce each other By comparisons between SHR and RHR an attempt was made to find out whether they differ quantitatively in terms of extent of structural changes for a given level of pressure increase

From a therapeutical point of view it is of interest to know to what an extent and how rapidly regression of cardiovascular hypertrophy may be accomplished if pressure is kept lowered This was studied by analysis of the cardiovascular changes at different intervals after reversal of renal hypertension (paper IV)

Moreover is it possible to prevent the development of renal

hypertension and its vascular changes by propranolol treatment as seems to be the case in SHR? The results may be of importance in evaluating the nature of the functional mechanisms initiating the two types of hypertension since the involvement of neurogenic mechanisms also in renal hypertension has recently been much discussed This problem was studied in paper V

METHODOLOGICAL PRINCIPLES

The experimental animal models

Animal models used earlier for comparison with human essential hyper tension all suffer from the drawback that they are induced by renal hormonal or nervous interferences in genetically normotensive animals However as already mentioned rat strains with spontaneous hypertension of polygenic background thus simulating the situation in essential hypertension of man have been developed more recently by Smirk and coworkers (e g Smirk and Hall 1958) and by Okamoto and his group (Okamoto and Aoki 1963) The latter strain has been used in this laboratory

Spontaneously hypertensive rats (SHR)

The SHR colony was produced by selective breeding of normal Wistar rats that showed a higher than average arterial pressure Successive brother-sister inbreeding of the colony was then performed and in 1969 when the first SHR group arrived in our laboratory they had reached the twentyfirst generation Brother-sister breeding was not consistently continued in order to avoid disturbing passenger pheno mena that tend to occur in too strict inbreeding Genetical studies suggest a polygenic background with an additive mode of inheritance of a relatively small number of major genetic elements (cf Okamoto 1972) Studies by Okamoto and coworkers (cf Okamoto 1972) strongly suggest that the SHR cardiovascular system is exposed to an increased centrally induced neurohormonal discharge which would constitute one of the key predisposing elements Further SHR exhibit exaggerated and often prolonged pressure and heart rate responses to alerting stimuli (Folkow Hallbäck and Weiss 1973) Also during anesthesia young SHR display a hyperkinetic hemodynamic pattern (Pfeffer and Frohlich 1973) apparently resembling a mild defence reaction Thus the early hyper tensive phase in SHR closely resembles the early "borderline phase of essential hypertension in man (e g Brod 1963 Julius Pascual and London 1971) In general SHR display signs of an intensified activity not only of the sympathico-adrenal system but also of the ACTH corticoid and TSH-thyroid systems (cf Okamoto 1969 1972)

The SHR hypertension develops gradually with increasing age but already at 6 weeks of age blood pressure is significantly raised compared to normotensive control rats (NCR) of the same age At the age of 5-7 months blood pressure has reached the level of 170-180 mm Hg

although this varies somewhat with the substrain used Concerning pathological findings in SHR the incidence of myocardial lesions nephrosclerosis and malignant vascular lesions is very high in more advanced stages and increases remarkably with severe hypertension (> 200 mm Hg) (cf Okamoto 1972)

In summary SHR seem to be the best animal model so far for essential hypertension in man and seem to exhibit all its known cha racteristics (Okamoto 1972) Hence SHR of both sexes in the phase of established hypertension (7-8 months of age) were used for the hemodynamic studies exploring the entire systemic vascular bed (study I) and the renal vascular bed (study II)

Renal hypertensive rats (RHR)

In studies III IV and V renal hypertensive rats were used Renal hyper tension was induced in 6-7 week old normotensive male Wistar rats by placing a standardized silver clip on the left renal artery during ether anesthesia leaving the right kidney intact Sixtyfive to eighty per cent of the operated rats developed hypertension (≥ 150 mm Hg) within 3 weeks after operation Thus by this Goldblatt procedure a marked hypertension developed quite rapidly in contrast to the more gradual onset of primary hypertension in SHR Therefore these renal hypertensive rats (RHR) offered pa ticularly favourable circum stances for exploring how soon cardiovascular changes are established once a rapid fairly persistent functional pressure rise is provoked Since these rats were genetically normotensive they were also used for comparison with the primary SHR hypertension for evaluations as to whether SHR might have a genetical predisposition for developing a quantitatively more pronounced media and left ventricular hypertrophy RHR also offered possibilities to study the rate and extent of regression of the cardiovascula structural changes since an immediate reversal of the RHR hypertension occurred upon removal of the renal artery clip

Blood pressure and heart rate measurements

In study I and II before the technique for measuring blood pressure directly in the caudal artery during awake conditions was developed arterial pressure was measured via a polyethylene tube in one of the femoral arteries during Nembutal® anesthesia (3-4 mg/100 g body weight i p) The tube was connected to a Statham pressure transducer and pressure was recorded on a Grass Polygraph model 7 Nembutal® anesthesia is known to depress both ventilation and temperature regu- lation which must of course affect the condition of the experimental

animals A tracheal cannula was always inserted to permit free air ways before arterial pressure was measured under anesthesia and this procedure was found to usually increase arterial pressure some 20 mm Hg in SHR and NCR On the other hand control measurements during awake and anesthetized conditions showed that the addition of Nembutal® usually decreased arterial pressure in both SHR and NCR around 20 mm Hg This means that the pressures actually given in study I and II seem to be roughly the same as those recorded directly during awake resting steady state conditions In studies III IV and V arterial pressure was measured in the caudal artery during awake resting conditions which ought to be the best way of measuring the true resting arterial pressure The procedure was the following: The caudal artery was cannulated during brief ether anesthesia The animal was then allowed to wake up and the arterial pressure was recorded by a Statham pressure transducer during resting conditions Control experi ments showed that the pressure level remained very constant when re peated recordings were made for several hours after the brief ether anesthesia Also heart rate could be recorded via the catheter in the tail artery

General perfusion procedures

The following methods have been used: Study I: Perfusion of the entire systemic vascular bed (except the coronaries) in SHR and NCR Study II: Paired perfusion of the isolated renal vascular bed in SHR and NCR Study III IV and V: Paired perfusion of the isolated hind-quarter vascular bed in RHR and NCR (Fig 2)

For details concerning the respective procedures see the indivi dual papers Below some general principles and considerations con ing the perfusion technique and its evaluation will be outlined

Perfusion medium

By using artificial perfusions for studies of the resistance vessels neuro-hormonal influences are eliminated reversible differences in ion com position of the vascular walls are diminished and vasoactive substances are washed out In all experiments oxygenated Tyrode solution was used with 4 per cent (3 per cent in study II) of Ficoll (a synthetic polymer of sucrose and epichlorohydrin m w around 80 000 AB Pharmacia Uppsala Sweden) as colloid substitute The temperature of the perfusate was kept at 38°C in the perfusate container being about 35-36°C when reaching the preparation The osmotic pressure of the perfusate was around 300 mOsm/l and viscosity determined in a

Wells Brookfield Micro viscosimeter was about 1 0 to 1 2 cP at 37°C depending on whether 3 or 4 per cent Ficoll was used Further different batches of Ficoll were used in different series of experiments due to a limited supply which slightly affected both viscosity and effective colloid osmotic pressure and hence some of the measured parameters However since all experiments except those in study I were run as equally treated pairs i e one SHR (or RHR) in parallel with one matched NCR the two vascular beds were always exposed to exactly the same perfusate and perfusion conditions largely cancelling out accidental interferences by e g differences in viscosity temperature or composition of the perfusate On the other hand such interferences probably explain why e g the mean values of the various NCR groups in study III IV and V differ slightly Such unavoidable though minor differences only serve to illustrate the great advantage of using paired experiments in quantitative comparative studies of this type Further the maximal pressor responses will to a slight degree be affected by a somewhat varying edema formation (see study III IV and V) which moderately raises tissue pressure and correspondingly reduces transmural vascular pressure This factor is of course increasingly important towards the end of the experiments Since edema formation was usually more pronounced in NCR this source of error tends to slightly overestimate the NCR maximal pressor response compared with that of SHR and RHR

Pressure-flow relationships during maximal dilatation

The pressure-flow relationships during complete vascular smooth muscle relaxation were examined in all 5 studies The vasculo bed was considered to be maximally dilated when no further relaxation could be obtained and to ensure complete smooth muscle relaxation repeated injections of papaverine were regularly used until no further resistance decrease was obtained

In order to further secure that the vascular bed was really maximally dilated it was checked that perfusion pressure returned to the same control level after sudden increases of flow rate No signs whatsoever of autoregulation were observed

When maximal dilatation was ensured the pressure-flow elationships were examined by changing the flow rate in a random fashion while recording the perfusion pressures Perfusion pressure was except in occasional tests kept below 40 mm Hg (60 mm Hg in study II) in order to avoid rapid edema formation in these maximally dilated vascular beds Thereafter pressure-flow curves were constructed for each individual experiment From these curves mean pressure-flow curves were later deduced

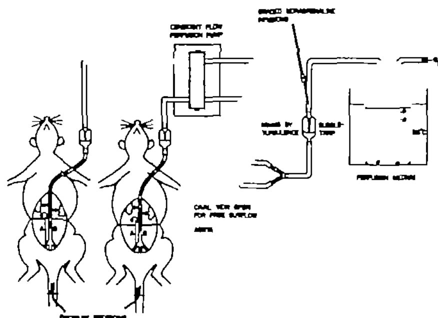


Fig 2 Paired perfusion of the isolated hindquarters of one renal hypertensive rat (RHR) and one normotensive control rat (NCR) as used in the studies III-V The perfusate emerges from a common container which is kept at 38°C and drained by a single tube coupled in series with a small mixing chamber that is steadily shaken The tube emerging from the mixing chamber branches into two tubes which after passing through a double Harvard perfusion pump are connected to the respective aortic cannulas After passage through the two parallel-coupled ar beds the perfusate is allowed to leave freely via the cut ferior caval veins Perfusion pressures are measured via the caudal arteries

Noradrenaline (NA) I fusion during constant flow conditions

This procedure was used in study II V and was identical in all studies except in study II where other flow values were used for the kidney Thus the pump was adjusted to deliver about 70 ml/min x 100 g to the renal vascular bed (study II) while the flow to the hindquarters was about 10 ml/min x 100 g (study III V) After having secured *complete smooth muscle relaxation with bolus injections of papaverine*

as mentioned above the perfusion was continued for some 15-20 minutes before NA infusion was started in order to completely wash out papaverine NA dissolved in the perfusion medium was then infused in a stepwise fashion from subthreshold up to supramaximal concentrations To induce definitely maximal pressor responses 0 5 - 1 0 mg of NA 10 IU of vasopressin and 100 - 150 mg of $BaCl_2$ were finally added The concentrations of vasoactive agents were always equal in the two preparations since the rats were of equal size equal flows per unit tissue mass were used and the injections were made proximally to a mixing chamber from which the two inflow tubes to the animals emerged (see Fig 2)

The perfusion pressure at maximal dilatation and the vasoconstrictor responses to NA vasopressin and barium chloride were then calculated Dose-response curves to NA were plotted for each paired experiment with μg of NA per ml perfusate on the (log) abscissa and perfusion pressure in mm Hg on the ordinate Since flow rate was constant the resistance responses can be expressed by the perfusion pressure responses

From these resistance curves the following key points were deduced and used when comparing the hemodynamic characteristics of the test animals to those of the controls :

1 Flow resistance at maximal dilatation representing the structurally determined average luminal size of the resistance vessels

2 The threshold concentration of NA i e the NA concentration raising flow resistance 25 per cent above that at maximal dilatation as deduced from the curve According to Poiseuille s law this would correspond to about 5 per cent shortening of the contractile elements if the influence of the wall/lumen ratio is neglected which seems justified since even in the fai ly thickwalled resistance vessels this ratio is low at maximal dilatation The NA threshold concentration represents the NA sensitivity of the smooth muscle cells

3 M_{50}, i e the NA concentration producing 50 per cent of the maximal NA pressor response M_{50} is not identical with ED_{50} as used in conventional dose-response curves for e g smooth muscle strips The reason is that the increasing wall/lumen ratio of constricting vessels in an accelerated way potentiates the luminal eduction for a given degree of smooth muscle shortening Further M_{50} is displaced to the left if one vessel is more thickwalled than the other even if thei smooth muscle sensitivity is equal (cf Fig 1 ight part)

4 The slope of the steep part of the resistance curve i.e the tangent of the angle which reflects the wall/lumen ratio as long as the degree

RESULTS AND COMMENTS

Structural changes of the total systemic vascular bed in SHR (paper 1)

In this study SHR in the phase of established hypertension were used to explore whether the adaptive vascular changes found in regional vascular beds in man with essential hypertension were present also in SHR and in that case if they were generalized throughout the systemic circulation Perfusion of the entire systemic vascular bed (except the coronaries) of SHR and NCR showed that even during maximal dilatation systemic resistance was raised in SHR almost in proportion to the raised arterial pressure As can be seen in Fig 3 which presents the mean pressure-flow curves of the maximally dilated systemic vascular beds flow resistance is throughout significantly increased in SHR compared to NCR The difference between SHR and NCR is particularly evident at the higher range of pressures which is in line with subsequent find ngs that the SHR resistance vessels are less distensible than the NCR ones (Hallbäck Lundgren and Weiss 1974)

These results are not in agreement with those of Haeusler and Finch (1972) which did not reveal any difference in resistance at maximal dilatation between SHR and NCR However there are two important differences between these two studies which may readily explain the apparently dive ging esults First in the present study 7 month old rats with well established hypertension were used while Haeusler and Finch used rats which being only 14 week old were in an earlier phase of hypertension where the structural vascular changes may not yet be fully developed Second in the present study flow was changed between 3 and 40 ml/mi x 100 g while in the study by Haeusler and Finch flow was not increased beyond 12 ml/min x 100 g As seen the present results however the difference in flow resistance is l at very low flows and distending pressures but increases at higher ows and perfusion pressures which reflects a reduced vascular distensibility in SHR

The prese t esults indicate that the earlier mentioned structural adaptation of the resistance vessels in primary hypertension is on the whole generalized throughout the systemic vascular bed and almost in proportion to the raised arterial pressure It follows that the raised flow resistance in SHR with established hypertension may to a great extent be accounted for by such a structural change, since it would reset the baseline for resistance vessel control to a higher level

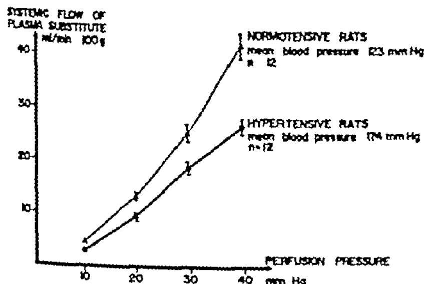


Fig 3 Mean pressure-flow curves from 24 perfusion experiments on the maximally dilated systemic vascular beds of 12 spontaneously hypertensive rats (SHR) and 12 normotensive control ats (NCR) Vertical bars indicate the standard error of the mean The two curves are significantly separated ($p < 0\ 001$)

Structural changes of the SHR renal vascular bed (paper II)

Paired perfusions of the renal vascular beds of 7 month old SHR (established phase of hypertension) and matched NCR showed that this particular ci cuit exhibited a lower esistance at maximal dilatation in SHR compared with NCR I other words the enal vascular bed differs in this espect from the enti e systemic vascular bed (study I) and the hindquarter vascular bed (Folkow t al 1970) in SHR as well as from the hand and forearm vascular beds i man with essential hypertension (Folkow Grimby and Thulesius 1958 Conway 1963 Sivertsson 1970) However the SHR renal resistance vessels also display a educed distensibility compared to those of NCR suggesting that the wider lumina ar associated with thicker vascular walls as in other hyperten i e circuits (cf H llback Lundgren and Weiss 1974)

Further Fig 4 illustrates that NA infusions during constant flow conditions evealed a steeper dose-response curve and an increased

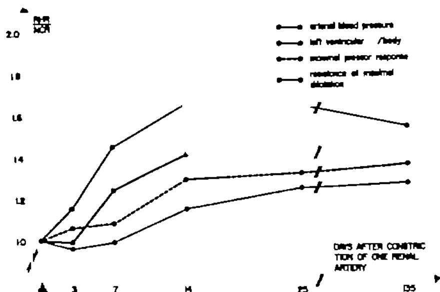


Fig 5 Time course and extent of adaptive cardiovascular changes in normotensive Wistar rats made hypertensive by renal artery constriction with days after artery constriction on the abscissa and on the ordinate the ratio between renal hypertensive rats (RHR) and normotensive control rats (NCR) concerning 1)arterial blood pressure (measured in the caudal artery during awake conditions), 2) left ventricular weight/body weight 3) maximal pressor response and 4) resistance at maximal dilatation (flow = 30 ml/ml x 100 g) Note that the adaptive hypertrophic changes of the left heart ventricle and the systemic resistance vessels are almost completed in RHR after 2-3 weeks The reduced pressure ratio at 135 days is due to a slight increase of NCR pressure

Rate and extent of development of adaptive cardiovascular changes in RHR (paper III)

In this investigation the extent and exact time course of cardiovascular structural adaptation to a rapid and fairly extensive rise in mean arterial pressure were studied after induction of renovascular hypertension in normotensive rats At different time intervals after renal artery constriction the hemodynamic characteristics of the RHR and NCR hind-

quarter vascular beds were explored from maximal dilatation up to maximal constriction Also the extent of left ventricular hypertrophy in RHR was examined The results revealed the presence of left ventricular hypertrophy in RHR already after one week closely followed by adaptive structural changes of the RHR resistance vessels Fig 5 illustrates the time course of these changes with days after renal artery constriction on the abscissa and on the ordinate is given the ratio RHR/NCR concerning arterial pressure left ventricular weight/body weight maximal pressor response and flow resistance at maximal dilatation The cardiovascular structural adaptation appears to be largely completed 2 3 weeks after operation since no further significant increase in blood pressure left ventricular hypertrophy or media hypertrophy appears to occur Thus compared to NCR the rats with established renal hypertension (25 days after operation) display in essence the same changes concerning their resistance curves as illustrated for SHR in the left part of Fig 1 i e 1) an increased resistance at maximal dilatation 2) a steeper slope of the resistance curve and 3) an increased maximal pressor response but no change in NA sensitivity (Fig 6) There is always a bigger resistance increase in RHR than in NCR for a given NA concentration which would he e produce largely the same extent of smooth muscle activation (as can be seen in Fig 6) - These results from RHR 25 days after operation are in agreement with findings on DOCA or renal hypertensive animals (Overbeck et al 1971 Bellin and Ziakas 1972 Haeusler and Finch 1972) with the exception that the latter investigators also reported an increased NA sensitivity of the resistance vessels in renal and DOCA hypertensive rats

It is seen in Fig 5 that the structural cardiovascular adaptation starts before the pressure rise is completed Thus this structural adaptation is evidently so rapid that e g its hemodynamic consequences in terms of both baroreceptor resetting and resistance rises for given smooth muscl activity lev ls are likely to become intertwined in time with the functional pressor influences which evidently act as trigger mechanisms for the structural changes This makes it probable that the very creation of the raised pressure is a matter of both functional and structural processes in close cooperation In man such structural changes are no doubt slower than in rats mainly because of the lower metabolic rate but the developme t of hypertrophic changes is likely to be quite rapid also in man I fact an i creased flow resistance even during maximal dilatation is observ d in the hand of young men (about 20 years old) with borderlin hypertension as compared to sex and age matched ormotensiv controls (Si ertsson Sannerstedt and Lundgren pr liminary results)

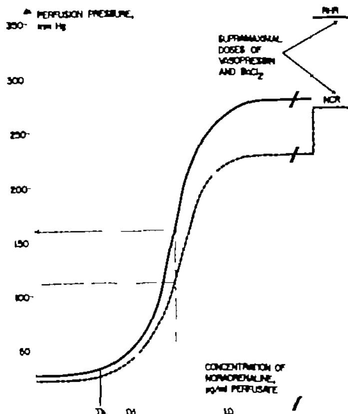


Fig 6 Mean resistance curves based on 12 constant flow perfusions of paired hindquarter vascular beds from renal hypertensive rats (RHR) 25 days after renal artery constriction (mean arterial pressure 180 ± 6 mm Hg) and normotensive control rats (NCR; mean arterial pressure 111 ± 3 mm Hg) showing the perfusion pressure (resistance) responses to increasing concentrations of noradrenaline (NA) from subthreshold up to supramaximal amounts Maximal pressor responses were ensured by huge doses of vasopressin and barium chloride NA concentration (µg/ml perfusate) is plotted along the abscissa on a log scale and perfusion pressure responses (mm Hg) along the ordinate Th denotes "threshold" i e the NA concentration producing 25 per cent increase of resistance above the state of maximal dilatation

Note the increased resistance already at maximal dilatation in RHR the enhanced steepness of the resistance curve and the increased maximal

pressor response while there is no difference in NA threshold dose between RHR and NCR The thin dotted lines illustrate how resistance to flow becomes higher in RHR than in NCR for the same NA concen tration i e the same degree of smooth muscle activation

---

135 days after operation there was a slight further increase in resistance at maximal dilatation and in steepness of the RHR dose response curve compared to RHR 25 days after operation However no further increase in maximal pressor response was noted These delayed changes might suggest a slight further increase in wall thickness that may not be due to an increase in smooth muscle tissue but in e g water or/and collagen content In fact an increased amount of collagen in the aorta has been demonstrated in long-term compared to short term renal hypertension (Wolinsky 1972) Concerning water logging (Tobian and Binion 1952 Tobian Olson and Chesley 1969 Tobian 1972) the present results reveal a significantly increased water content of the RHR aortic walls 135 days after operation compared to matched NCR and/or RHR 25 days after operation but no difference was found between RHR 25 days after operation and their matched NCR From these results which however are obtained f om the aorta it seems possible that both collagen invasion and some water logging may con tribute somewhat to the wall thickeni g also in the resistance vessels in later phases of hypertension though the hypertrophic adaptation seems to be by far the most rapid extensive and therefore hemo- dynamically important structural change in these vessels

The present results illustrate in principle that the cardiovascular structural adaptation to increased pressure occurs not only in animals genetically predisposed to hypertension lik SHR but also in gene tically normotensive animals in which hypertension is induced by inter ference with e g the enal blood supply (RHR) However even if the same kind of cardiovascular change i present in both types of hyper tension, this does not exclude the possibility of a quantitative difference i e the cardiovascular structures in primary hypertension may respond with somewhat more pronounced changes in design when exposed to a given pressur load In case such a difference really exists it is not necessarily inherent in the cardiovascular tissues per se, it may as well be a consequence of some genetically linked difference in e g hormo- nal mechanisms that f cilitate the development of hypertrophi changes

A comparison has been made between SHR (8 months ld) and RHR (135 days after operation, 6 months old) where the hypertensive st t is of approximat ly the same duration The arterial pressure levels were related to left entricular weight and to maximal contractil strength

of the resistance vessels (Folkow et al 1973) In order to investigate to what an extent the hypertrophic changes were in balance with the load to which heart and resistance vessels were exposed When comparing SHR and RHR in this way the hypertrophic changes appeared to be somewhat more pronounced in SHR than in RHR if resting arterial pressure can be considered as a reflection of the average pressure load This is however one of the problems in hypertensive research in general because it is in reality not known exactly what the average pressure is during days and weeks since this would call for long-term continuous pressure measurements in normal daily life No doubt the present results may support the view that SHR display a more pronounced cardiovascular hypertrophy than RHR for a given load but to prove this is hardly possible at present It might for example also be so that the true average pressure in SHR is in reality higher than the measured resting pressure level would indicate while this latter level better corresponds to the average pressure in RHR Thus SHR might display pronounced and frequent pressure enhancements as a result of transient neurohormonal discharges in daily life superimposing an extra pressure load which may be far less pronounced in RHR No doubt the SHR pressor responses to acute alerting stimuli are more pronounced than in RHR (Hallbäck and Folkow 1974) If this latter alternative is of relevance it would however in itself imply an important and interesting difference between primary and secondary hypertension

Studies where renal hypertension has been provoked in both young SHR and young NCR are in progress as an attempt to shed further light on the problem whether the cardiovascular changes are more pronounced in primary hypertension than in renal hypertension but it still too early to draw any conclusions In any case it appears as this structural autoregulation is so pronounced in SHR as to largely explain the raised resistance during rest, while this is hardly the case in RHR where functional excitatory influences evidently must contribute more strongly to maintain the raised resistance and pressure levels However the question if the hypertrophic cardiovascular changes are more pronounced in SHR than in RHR is far from settled and more studies are needed to solve this problem

Regression of hypertensive cardiovascular changes upon reversal of renal hypertension in rats (paper IV)

To study how rapidly and to what extent regression of the hypertrophic cardiovascular changes in renal hypertension can occur the renal artery clip was removed 3–4 weeks after clipping Arterial pressure had

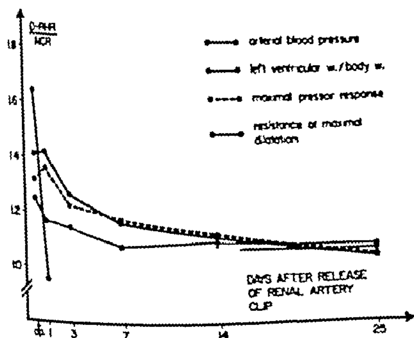


Fig 7 Time course and extent of regression of the hypertensive changes in cardiovascular design after reversal of renal hypertension Days after declipping are plotted along the abcissa and along the ordinate the ratios between renal hypertensive rats (RHR) before and after (D-RHR) declipping and their normotensive cont ol rats (NCR) concerning 1) arterial pressure 2) left ventricular weight/body weight 3) maximal pressor response and 4) flow resistance at maximal dilatation (Flow = 30 ml/min x 100 g) Thus the cardiovascular system still displays structural hypertensive changes one day after declipping but rapid regression then occurs with normalization after about 3 weeks

---

then stabilized at about 180 mm Hg and according to the previous study the structural adaptation was fully de eloped Pol ed hindquarter perfusions were performed on one declipped RHR (D-RHR) and one normotensive control rat (NCR) 1 3 7 14 and 25 days after releasing the clip thus exploring the differences between them concerning resistance vessel design On the same occasions the egression of cardiac hypertrophy was followed by measuring left ventricular weights

Blood pressure was normalized already after one day but no signi

ficant regression of the hypertensive structural changes could be traced neither 1 nor 3 days after unclamping Then however both the cardiac and vascular hypertrophic changes rapidly diminished and were largely eliminated after 3 weeks as illustrated in Fig 7 In this Fig days after release of the renal artery clip are given along the abscissa while the ordinate gives the ratios between D-RHR and NCR concerning arterial pressure left ventricular weight/body weight maximal pressor response and resistance at maximal dilatation Resistance at maximal dilatation appears to be the parameter most rapidly normalized, i e almost within 1 week while the increased steepness of the D-RHR resistance curve the maximal pressor response and the left ventricular weight were largely normalized in 3 weeks indicating a regression of media and cardiac hypertrophy

The early decrease of resistance at maximal dilatation implies only some 4-5 per cent increase in average internal radius negligibly affecting wall tension according to Laplace s law This effect on wall tension is completely overcome by the far bigger fall in transmural pressure However this nevertheless illustrates that the internal radius can change relatively independently of wall thickness Evidently wall thickness adjusts more or less precisely to wall tension while internal radius might also respond to other elements such as the local chemical environment as determined by the balance between tissue metabolism and its blood supply

The present results are in agreement with studies on cat and SHR where pressure reductions after arterial obstructions or hypotensive drug treatment induced rapid changes of resistance vessel design (Folkow and Sivertsson 1968 Folkow et al 1971 b Weiss 1974 Weiss and bäck 1974) Further Immunosympathectomy in newborn SHR or aortic in young SHR largely prevents the development of the mentioned hypertensive vascular changes (Folkow et al 1971 a Folkow et al 1972) Together these studies suggest that the hypertrophic cardiovascular changes mainly represent a regional structural adaptation to the average pressure load

Likewise morphological and chemical studies of the rat thoracic aorta (Wolinsky 1971) reveal a considerable decrease of the smooth muscle component after reversal of renal hypertension However in these studies the duration of hypertension was longer than in the present study (10 weeks compared with 3-4 weeks) and there was no complete regression of media thickness at least not in male rats The reason was mainly that there was also a considerable increase of elastin and collagen in the aortic wall and these two substances showed very poor regression upon pressure reduction Such elements may increase also in the resistance vessels proper with time even though their rela

tive proportions are here normally much smaller However once a substantial collagen invasion has occurred also in the resistance vessels its regression is likely to be as sluggish as in the aorta This may explain why Tobian Olson and Chesley (1969) did not observe any decrease in mesenteric arteriolar wall thickness after reversal of long standing renal hypertension

The results of the present study together with those of e g Tobian Olson and Chesley (1969) Wolinsky (1971 1972) and Weiss (1974) illustrate the advantage of early antihypertensive therapy before accumulation of elastin and collagen occurs since the earlier established hypertrophic changes seem to disappear completely and rapidly Once the less reversible changes, e g accumulation of collagen, are established both resetting of the baroreceptor mechanisms to normotensive levels (cf Aars 1969) and readjustment of resistance vessel dynamics would be far more difficult to achieve by antihypertensive treatment

The present results confirm the findings by e g Byrom and Dodson (1949) Floyer (1955) Ledingham and Cohen (1962) Liard and Peters (1970) and Funder et al (1970) Thus blood pressure in relatively ea ly renal hypertension in rats is rapidly normalized after release of the renal artery constriction This occurs despite the presence of considerable hypertrophic cardiovascular changes According to Ledingham (1971) this rapid pressure normalization is the result of a sharp reduction in cardiac output while resistance further increases If anything at least in short term measurements (6 hours) The hemodynamic situation is however extremely complicated; for example the sudden overperfusion of the earlier obstructed low pressure kidney may initiate complex neurohormonal shifts where among other possibilities depressor substances from the kidney in the form of prostaglandins (e g Tobian 1972) etc may be of importance Further the neurogenic tonic influences might become subnormal as a result of the suddenly changed renin-angiotensin equilibrium However very little is so far known about these events and thei background and more penetrating studies are obviously needed

If in well established renal hypertension the clipped kidney is instead extirpated the hypertensive state tends to prevail (Wilson and Byrom 1941 Pickering 1945 Floyer 1955 Funder et al 1970) indicating extrarenal pressor mechanisms However extirpation of the ischemic kidney after short term hypertension (0-8 days) brings bout an initial decrease l blood pressure (Pickering 1945) It is possible that the presently studied structural autoregulation of the resistance vessels may at least partly account for the extrarenal pressor mechanisms On the other hand preliminary results from this laboratory

reveal an immediate normalization of blood pressure when the ischemic kidney is extirpated 4 weeks after renal artery constriction

Obviously the results in this field are still confusing and in part contradictory calling for more penetrating studies of e g neurogenic control the balance between cardiac output resistance and capacitance vessel control etc The sudden release of the renal artery constriction or extirpation of the clipped kidney with a consequent loss of power ful humoral drive mechanisms are likely to induce gross though transient changes in neurohormonal cardiovascular control If so the consequent depressor effects would counteract the impact of already established structural changes and allow for their gradual elimination Thus whatever the mechanism behind the normalization of blood pressure in early renal hypertension the reduced pressure level will lead to a rapid and complete regression of the structural cardiovascular changes and hence to a stabilization of the normotensive state

Propranolol treatment in RHR (paper V)

It was explored whether preventive treatment with β-receptor antagonists would interfere with the developme t of renal hypertension and the hypertrophic vascular changes first because neurogenic elements and cardiac output increases have been much discussed also in early renal hypertension (cf Page and McCubbin 1968 Guyton and Coleman 1969 Ledingham 1971) and second because such treatment effectively prevents the progress of SHR hypertension (Folkow Lundgren and Weiss 1972 Weiss Lundgren and Folkow 1974)

The results of the present study indicate that in contrast to the situation in young SHR renal hypertension develops just as ly despite preventive treatment with propranolol in concentrations would efficiently block the adrenergically mediated renin secretion edykeen et al 1970 Bühler et al 1972 Michelakis and McAllister ,72) as well as the adrenergic control of the heart and thereby de crease the cardiac output (Frohlich et al 1968 Ulrych et al 1968) However the exact mechanisms of β-adrenergic receptor antagonists are not yet known and it is possible that they exert one of their main effects via the central nervous system (Sannerstedt 1974) Paired hindquarter perfusions revealed the same degree of hypertensive structural changes as in untreated RHR controls Further prolonged propranolol treatment in rats with already established renal hypertension did not affect arterial pressure Therefore the present results do not suggest any major importance of the adrenergically mediated increases of cardiac output or/and enin release neither for the development nor for the maintenance of renal hypertension The difference between these

results in RHR and those in early SHR hypertension also supports the view that the initiating mechanisms must be different in these two types of hypertension

GENERAL DISCUSSION

Adaptive structural changes of the resistance vessels mainly in the *form of an increased media thickness associated with a luminal reduction* have been demonstrated in regional vascular beds in man with essential hypertension as well as in SHR (cf Folkow et al 1973) Like left ventricular hypertrophy and similar changes in larger arteries this phenomenon may be considered as a basically normal response of mesodermal tissues to increases in functional load thus occurring in any type of sustained increase of average pressure the reverse process taking place upon pressure reductions These secondary structural adjustments of the cardiovascular system occur so rapidly (study III) that their hemodynamic consequences in terms of e g the resistance rise for a given smooth muscle activity level or the resetting of baroreceptors (cf Aars 1969) are likely to become intertwined in time with the functional pressor influences which serve to trigger the structural adaptation Hence these changes are likely to contribute not only to the maintenance but also to the very creation of a chronic hypertensive state In rats they are almost completed in 3 weeks though in man the slower metabolism is likely to imply a considerably longer period However even if the development of hypertrophic adaptation is slower in man it is still a relatively rapid process considering the gradual onset of essential hypertension and the far longer life span in man

The structural vascular changes are generalized occurring in the entire systemic vascular bed as shown in study I However when comparing different systemic circuits they seem to differ somewhat in of luminal dimensions at maximal dilatation (study II) Thus the renal circuit displays a decreased flow resistance during maximal dilatation at least at relatively low transmural pressures while the vascular bed of e g the skeletal muscles and the average of the systemic vascular bed then display an increased resistance This difference might be a reflection of corresponding differences in basal vascular tone precapillary smooth muscle tone being normally very low in the kidneys but quite high in e g skeletal muscle implying that the structural vascular adaptation in such circuits is initiated from quite different levels of functionally determined wall/lumen ratios In this respect the situation in the renal resistance vessels is related to that in most conduit arteries where smooth muscle tone is usually low and where the adaptive wall thickening is also usually associated with an increased inner radius However common to all vessels exposed to an increased average pressure load seems to be the increased wall thickness *leading to a raised wall/lumen ratio (see also Furuyama*

1962 Suwa and Takahashi 1971)

Changes of this general nature are indeed biologically most appropriate, e g for inducing structural adaptations also in the normal vascular bed Thus vessels exposed to enhanced transmural pressures as a result of hydrostatic influences, e g the veins of the lower limbs, tend to increase their wall thickness (Jones and Dale 1958 Svejcar et al 1962) The same is true in pulmonary hypertension (Ferguson and Varco 1955) With respect to the resistance vessels these locally induced structural adjustments may be called structural autoregulation as a more slowly developed correlate to the acute functional autoregulation displayed by most resistance vessels as a local response to sudden pressure changes Like this process it implies however a type of positive feedback mechanism and therefore a potential risk factor particularly when it affects the systemic vascular bed as a whole The reason is that it tends to set the pressure-resistance equilibrium to a higher level and thereby increasing the chances of degenerative and lesional changes and thus true disease

An important question is if animals with primary hypertension (SHR) are perhaps genetically more prone to respond with cardiovascular hypertrophy to a given increase in pressure load than those with secondary hypertension (RHR) Even a modest quantitative difference in this respect is important since it may in the long run have considerable hemodynamic consequences because of the tendency of the functional and structural factors to reinforce each othe with respect to resistance vessel control From the present study it appears as if the extent of structural change of heart and resistance vessels may be somewhat more pronounced in SHR than in RHR for a given level of resting arterial pressure As a possible parallel it should be recalled that patients with essential hypertension have a prevalence of mesomorphic body build (Robinson and Brucer 1940) which with espect to heart and blood vessels might imply a greater tendency to respond structurally to a pressure load as may be the case in SHR However as discussed earlier this problem is very difficult to settle and the evidence so far should rathe be considered as indicative

Another important question is: What initiates these structural changes of heart and resistance vessels? Primary and renal hypertension will here be discussed separately since fundamental differences evidently exist between these two types of hypertension concerning the nature of the functional initiating mechanisms

Primary hypertension

The strong hereditary element in this important disorder of regulation is well known and seems to be made up of several genetically linked factors both in man and in rats (Pickering 1968 Okamoto 1972) As previously mentioned the observed structural cardiovascular changes *are secondary in nature though rapidly established and perhaps particularly* easy to induce in subjects predisposed for hypertension The establishment calls for trigger factors in terms of functional increases of the average pressure load continuous or intermittent in nature It is however unlikely that such a pressure load would be of renal origin in SHR (see Okamoto 1972) and in study II it is shown that there is nothing to indicate any structurally based Goldblatt mechanism in early SHR hypertension

It has been proposed that primary hypertension in man might be due to a complex interaction between centrally elicited increases of arterial pressure and cardiac output with a gradual structural adaptation of heart and precapillary vessels to such functionally elicited pressure increases (Folkow 1960) The characteristic hypothalamic defence reaction (e g Eliasson et al 1951) conveying most such responses elicited by psychogenic stimuli is present throughout the animal species including man (e g Folkow 1960 Brod et al 1962 Brod 1963 Charvat, Dell and Folkow 1964 Folkow and Rubinstein 1966 Henry Meehan and Stephens 1967 Folkow and Neil 1971) In fact weak but often repeated stimulations of the hypothalamic defence area in rats result in a moderate but fairly sustained hypertension (Folkow and Rubinstein 1966) as is the case also when mice and monkeys are exposed to environmental stress (Henry Meehan and Stephens 1967 Herd et al) Also in man the characteristic defence reaction induced by ..strain of daily life can lead to often considerable although ..sient increases in cardiac output and blood pressure (Brod 1960 ..3 Hinma Engel and Bickford 1962 Pickering 1968 Bevan Honour .. Stott 1969) In fact early labile stages of essential hypertension usually display a cardiovascular pattern closely mimicking a mild defence reaction (e g Brod 1963 Sannerstedt 1966 Julius Pascual and London 1971) Thus it seems as the central discharge pattern to ordinary alerting situations is accentuated in subjects developing essential hypertension (e g Hines and Brown 1933 Kalis et al 1957 Folkow 1960 Charvat Dell and Folkow 1964 Nestel 1969 Lorimer et al 1971

A long series of studies by Okamoto s group (cf Okamoto 1969 1972) provides evidence of an enhanced neurohormonal discharge to the cardiovascular system in SHR Further also young prehypertensive

SHR display an inherent evidently genetically linked hyperreactivity of the autonomic centers which during alertness and mental stress mediate increased changes in cardiovascular function (Hallbäck and Folkow 1974) Even if largely intermittent in nature such influences may well serve to initiate structural ca diovascular adaptation as discussed above Such an inherent hyperreactivity of central autonomic structures will also be greatly influenced by the environmental situation being further potentiated by stress thus implying a complex interaction between genetic and environmental factors

The importance of neurogenic mechanisms for initiation of hyper tension in SHR is also evident by the effects of immunosympathectomy in newborn SHR (Clark 1971 Folkow et al 1972) and of prolonged treatment with β-adrenergic receptor antagonists and other sympathicolytic drugs in young SHR (Weiss 1974 Weiss Lundgren and Folkow 1974) All these procedures interfere more or less drastically with the develop ment of spontaneous hypertension and the associated vascular changes

In other words both in SHR and in man with essential hyper tension there may be a combination of increased central neuro-hormonal reactivity and secondary although rapidly developed structural cardio vascular changes the latter being a response to the enhanced average pressure load Both of these factors are per se perfectly normal mechanisms which however may for genetical reasons be somewhat enhanced in extent in SHR and perhaps also in man with essential hypertension However the situation is in all probability far more complex than so because also other elements are likely to be involved and the balance between them may vary from individual to indi idual at least in man where the genetic background is far more heterogenous than in the inbred SHR

Renal hypertension

The background of the initial increase in blood pressure in renal hyper tension is still far from clear despite great experimental efforts No doubt renal humoral mechanisms are of primary importance but they may to a great extent exert their cardiovascular effects via neurogenic links (Dickinson and Yu 1967 Ueda et al 1969 Joy 1971 Ferrario Gildenberg and McCubbin 1972) as outlined earlier In initial phases there is often a transie t increase of cardiac output noted by many investigators (cf Ledingham 1971 Bianchi et al 1972) However this proposed increase in cardiac output is then not only a matter of the adrenergic myocardial innervation since prolonged treatm...

the adrenergic β-receptor antagonist propranolol did in no way interfere with the development of renal hypertension or the hypertensive *vascular changes (study V) The initiating renal mechanisms are evi* dently capable of inducing hypertension also via other links of the autonomic innervation in cooperation with e g direct influences on the vessels interferences with the sodium balance via aldosteron etc In fact the importance of the sympathetic nervous system for the development of renal hypertension is still not clear Thus Finch and Leach (1970) concluded that renal as well as DOCA hypertension can develop even though most of the sympathetic nervous system has been destroyed while Grewal and Kaul (1971) and Ayitey-Smith and Varma (1970) concluded that at least some aspects of the sympathetic control of the cardiovascular system are essential for the development of renal hypertension

Part of the renin secretion (cf Davis 1973) is mediated via adrenergic β-receptors (Vandongen Peart and Boyd 1973) but probably not that part which is induced by regional hypotension and relative ischemia of the kidney (e g Blaine and Davis 1971) The fact that propranolol treatment which seems to inhibit the neurogenic control of renin secretion in no way prevents the development of renal hypertension (study V) indicates that the other local mechanism for renin secretion is the by far dominating one for eliciting renal hypertension Further immunization against angiotensin does not prevent the development of renal hypertension (e g Eide and Aars 1970 Johnston Hutchinson and Mendelsohn 1970 MacDonald et al 1970 Eide 1972) indicating either that the kidneys possess other means for raising arterial pressure when their perfusion is threatened or the immunization was not capable of surpressing all angiotensin e g the facilitating effect on sympathetic discharge via bulbar ...ures

The fact that pressure promptly falls to normal or even subnormal ...vels after release of the renal artery constriction (and even sometimes after extirpation of the ischemic kidney), despite the presence of cardiovascular hypertrophic changes (study IV) indicates that cardiac output or/and vascular tone must then become subnormal The sudden elimination of renal humoral excitatory influences perhaps associated with an excess release of depressor humoral agents like prostaglandins etc from medullary interstitial cells (e g Tobia 1972) along with the over perfusion of the earlier ischemic kidney may here be of great importance Such a subnormal vascular tone and neurogenic drive on the system will if prolonged enough not only normalize blood pressure even if extensive structural cardiovascular changes are present but also induce regression of these changes However such a proposed sequence of

mechanisms is difficult to follow in detail experimentally For such reasons very little is so far known about the true events which what ever their nature are of great importance for understanding renal hypertension

Regression of cardiovascular changes

The present study (IV) has shown that a rapid regression of cardiac as well as media hypertrophy occurs after normalization of blood pressure at least in early renal hypertension Thus all cardiovascular changes are eliminated after 3–4 weeks of normotension The same general time course is seen in cats or in SHR when only regional hypotension is induced (Folkow and Sivertsson 1968 Weiss and Hallbäck 1974) Further intensive treatment of SHR with hypotensive drugs leads to considerable regression of these changes already after 5 weeks although this regression appears to become slower and less complete the more advanced the hypertensive state (Folkow et al 1971 b Weiss 1974) The slower and less complete regression of the vascular changes in longterm hypertension may reflect that the rapidly established smooth muscle hypertrophy in the resistance vessels is gradually superimposed by or/and transformed into structural changes of a more degenerative nature such as collagen invasion which are less liable to exhibit regression than smooth muscle hypertrophy A parallel may here be drawn to the aorta where it has been observed that increased amounts of collagen and elastin are formed in long-term hypertension and that these wall elements show far less regression than the smooth muscles upon reversal of renal hypertension (Wolinsky 1971 1972)

Together these results illustrate the advantage of an early induction of antihypertensive therapy when the structural changes are still mainly in the phase of muscle hypertrophy Thus if blood pressure is normalized in this early phase the hypertrophic changes of the resistance vessels and also of the left ventricle and probably large arteries are likely to disappear completely and rapidly The tendency of a vicious circle inherent in the potentiation between adaptive hypertrophic changes and functional excitatory influences in terms of resistance vessel responses will then also be i terrupted Once more persistent changes have been added such as collagen invasion in heart arteries and resistance vessels these changes also increasing wall thickness would tend to remain decreasing the chances for inducing a true reversal of the hypertension state Thus the resistance vessels would then still tend to exhibit exaggerated luminal reductions upon smooth muscle contractions and the stiffer walls at the site of the arterial baroreceptors would interfere with thei resetting to the normal pressure range (cf Aars 1969)

SUMMARY OF THE PRESENT RESULTS

The results obtained in these comparative hemodynamic studies on spontaneously hypertensive rats (SHR) renal hypertensive rats (RHR) and normotensive control rats (NCR) can be summarized as follows:

I Adaptive structural changes of the resistance vessels are largely generalized throughout the systemic vascular bed in SHR They seem to be largely in proportion to the raised arterial pressure but are evidently mainly confined to the precapillary vascular sections to judge from still more recent findings

II No structurally based Goldblatt mechanism seems to be involved in the establishment of SHR primary hypertension Thus the SHR renal vascular bed displays a reduced resistance to flow during maximal dilatation In contrast to most other systemic circuits in SHR However like the situation in these other systemic circuits the SHR renal resistance vessels are characterized by an increased wall/lumen ratio to judge from their hemodynamic characteristics

III The cardiovascular structural adaptation to increases in pressure load is qualitatively equal in rats with genetically linked hyper tension (SHR) and in genetically normotensive rats (RHR) but might be quantitatively somewhat more pronounced in SHR than in RHR The process is a very rapid one with respect to the development of media and left ventricular hypertrophy being completed about 3 weeks after initiation of renal hypertension in rats Vascular water logging appears to occur only in late phases of hypertension and seems even then to be of far less hemodynamic significance than the structurally based increase of wall thickness

IV After reversal of early renal hypertension the hypertrophic cardio vascular changes are hardly significantly changed during the very first few days Then however a rapid gradual regression occurs so that a normalization of the design of heart and resistance vessels appears to occur after 3–4 weeks of normotension

V Propranolol treatme t effectively interfering with the development of SHR hypertension has no similar preventive effects either on the development of renal hypertension or the hypertensive vascular changes Further no hypotensive effect of prolonged propranolol treatment was found on already established renal hypertension

The mentioned findings I SHR RHR and NCR are discussed with

particular reference to the interactions between functional and structural changes affecting resistance vessel control in primary and secondary hypertension

ACKNOWLEDGEMENTS

I am deeply grateful to my teacher Professor Björn Folkow for his guidance advice and invaluable help throughout this study

I also wish to thank my co-workers Margareta Hallbäck and Lilian Weiss for co-operation and stimulating discussions

Thanks are also due to Gunnel Andersson and Ulla Axelsson for the most excellent technical assistance to Kerstin Lundgren and Sylvia Palmquist for skilful typing of the manuscripts to the photographer Thomas Palm and the animal keeper Gösta Magnusson for valuable help

My thanks are also due to Dorothy Stage for revising part of the English text in this summary

This investigation was supported by grants from the Swedish Medical Research Council (No 14X-16) from the Faculty of Medicine University of Göteborg from Svenska Sällskapet för Medicinsk Forskning and from the Swedish National Association against Heart and Chest Diseases

AB Hässle Mölndal Sweden generously covered part of the expenses for technical assistance Thanks are also due to AB Pharmacia Uppsala Sweden for generous supply of Ficoll and to ICI Ltd Great Britain for gifts of propranolol

AARS H Relationship between aortic diameter and aortic baroreceptor activity in normal and hypertensive rabbits Acta physiol scand 1969 75 406-414

ALLBUTT T C Diseases of the arteries including angina pectoris London Macmillan and Co Ltd 1915

ASSAYKEEN T A P L CLAYTON A GOLDFIEN and W F GANONG Effect of α and β-adrenergic blocking agents on the renin response to hypoglycemia and epinephrine in dogs Endocrinology Springfield 1970 87 1318 1322

AYITEY-SMITH E and D R VARMA An assessment of the role of the sympathetic nervous system in experimental hypertension using normal and immunosympathectomized rats Brit J Pharmacol 1970 40 175-185

BAEZ S Simultaneous measurements of radii and wall thickness of microvessels in the anesthetized rat Circulat Res 1969 25 315-329

BASCH S von Über late te Arteriosclerose und deren Beziehung zu Fettleibigkeit Herzerkrankungen und anderen Begleit erscheinungen Wien Urban & Schwartzenberg 1893

BEILIN L J and G ZIAKAS Vascular reactivity in post-deoxycorticosterone hypertension in rats and its relation to irreversible hypertension in man Clin Sci 1972 42 579 590

BELLO C T R W SEVY and C HARAKAL Varying hemodynamic patterns in essential hypertension Amer J med Sci 1965 250 24-35

BEVAN A T A J HONOUR and F H STOTT Direct arterial pressure recording in unrestricted man Clin Sci 1969 36 329-344

BIANCHI G E BALDOLI R LUCCA and P BARBIN Pathogenesis of arterial hypertension after the constriction of the renal artery leaving the opposite kidney intact both in the anaesthetized and in the conscious dog Clin Sci 1972 42 651-664

BLAINE E H and J O DAVIS Evidence for a renal vascular mechanism in renin release: New observations with graded stimulation by aortic constriction Circulat Res 1971 28 29 Suppl II II:118 II:126

BRIGHT R Tabular view of the morbid appearances in 100 cases connected with albuminous urine With observations Guy s Hosp Rep 1836 1 380–400

BROD J Haemodynamic responses to stress and its bearing on the haemodynamic basis of essential hypertension In The pathogenesis of essential hypertension Proc Joint WHO-Czechoslov Cardiol Soc Symp State Med Publ House Prague 1960 256–264

BROD J Haemodynamic basis of acute pressor reactions and hypertension Brit Heart J 1963 25 227–245

BROD J V FENCL Z HEJL J JIRKA and M ULRYCH General and regional haemodynamic pattern underlying essential hypertension Clin Sci 1962 23 339–349

BUHLER F R J H LARAGH L BAER E D VAUGHAN and H R BRUNNER Propranolol inhibition of renin secretion New Engl J Med 1972 287 1209 1214

BYROM F B and L F DODSON The mechanism of the vicious circle in chronic hypertension Clin Sci 1949 8 1–10

CHARVAT J P DELL and B FOLKOW Mental factors and cardiovascular diseases Cardiologia 1964 44 124–141

CHILD C G Observations on the pathological changes following experimental hypertension produced by constriction of the renal artery J exp Med 1938 67 521–531

CLARK D W J Effects of immunosympathectomy on development of high blood pressure in genetically hypertensive rats Circulat Res 1971 28 330–336

CONWAY J A vascular abnormality in hypertension A study of blood flow in the forearm Circulation 1963 27 520–529

DAVIS J O The control of renin release Amer J Med 1973 55 333–350

DICKINSON C J and R YU Mechanisms involved in the progressive response to very small amounts of angiotensin in conscious rabbits Circulat Res 1967 21 Suppl II II:157 II:163

DJOJOSUGITO A M B FOLKOW B ÖBERG and S WHITE A comparison of blood viscosity measured in vitro and in a vascular bed Acta physiol scand 1970 78 70–84

DOYLE A E Vascular reactivity in human hypertension New Zeald med J 1968 67 295-303

DRURY D R The production by a new method of renal insufficiency and hypertension in the rabbit J exp Med 1938 68 693 701

EIDE J Renovascular hypertension in rats immunized with angiotensin II Circulat Res 1972 30 149-157

EIDE J and H AARS Renal hypertension in rabbits immunized with angiotensin II Scand J clin Lab Invest 1970 25 119 127

ELIASSON S B FOLKOW P LINDGREN and B UVNÄS Activation of sympathetic vasodilator nerves to the skeletal muscles in the cat by hypothalamic stimulation Acta physiol scand 1951 23 333 351

EWALD C.A Ueber die Veränderungen kleiner Gefässe bei Morbus Brightii und die darauf bezüglichen Theorien Virchows Arch path Anat 1877 71 453-499

FERGUSON D J and R L VARCO The relation of blood pressure and flow to the development and regression of experimentally induced pulmonary arteriosclerosis Circulat Res 1955 3 152 158

FERRARIO C M P L GILDENBERG and J W McCUBBIN Cardiovascular effects of angiotensin mediated by the central nervous system Circulat Res 1972 30 257 262

FERRARIO C M I H PAGE and J W McCUBBIN Increased cardiac output as a contributory factor in experimental renal hypertension I dogs Circulat Res 1970 27 799-810

FINCH L and G D H LEACH The contribution of the sympathetic nervous system to the development and maintenance of experimental hypertension in the rat Brit J Pharmacol 1970 39 317-324

FLOYER M.A Further studies on the mechanism of experimental hypertension in the rat Clin Sci 1955 14 163-181

FOLKOW B Structural myogenic humoral and nervous factors controlling peripheral resistance Hypotensive Drugs 1956 Pergamon Press London 163-174

FOLKOW B Haemodynamic responses to cortical and hypothalamic stimulation In The pathogenesis of essential hypertension Proc joint WHO-Czechoslov Cardiol Soc Symp State Med Publ House Prague 1960 247 255

FOLKOW B G GRIMBY and O THULESIUS Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta physiol scand 1958 44 255-272

FOLKOW B M GUREVICH M HALLBACK Y LUNDGREN and L WEISS The hemodynamic consequences of regional hypotension in spontaneously hypertensive and normotensive rats Acta physiol scand 1971a 83 532 541

FOLKOW B M HALLBACK Y LUNDGREN R SIVERTSSON and L WEISS Importance of adaptive changes in vascular design for establishment of primary hypertension studied in man and in spontaneously hypertensive rats Circulat Res 1973 32 33 Suppl I I:2-I:13

FOLKOW B M HALLBACK Y LUNDGREN and L WEISS Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats Acta physiol scand 1970 80 93 106

FOLKOW B M HALLBACK Y LUNDGREN and L WEISS The effect of intense treatment with hypotensive drugs on structural design of the resistance vessels in spontaneously hypertensive rats Acta physiol scand 1971b 83 280-282

FOLKOW B M HALLBÄCK Y LUNDGREN and L WEISS The effects of immunosympathectomy on blood pressure and vascular reactivity in normal and spontaneously hypertensive rats Acta physiol scand 1972 84 512-523

FOLKOW B M HALLBACK Y LUNDGREN L WEISS I ALBRECHT and S JULIUS Analysis of design and reactivity of series coupled vascular sections in spontaneously hypertensive rats (SHR) Acta physiol scand 1974 In press

FOLKOW B M HALLBACK and L WEISS Cardiovascular responses to acute mental stress in spontaneously hypertensive rats Clin Sci 1973 45 131s 133s

FOLKOW B Y LUNDGREN and L WEISS The effect of prolonged propranolol treatment on blood pressure and structural design of the resistance vessels in young spontaneously hypertensive rats (SHR) Acta physiol scand 1972 84 8A 9A

FOLKOW B and E NEIL Circulation, Oxford University Press Oxford New York 1971

FOLKOW B and E H RUBINSTEIN Cardiovascular effects of acute and chronic stimulations of the hypothalamic defence area in the rat Acta physiol scand 1966 68 48 57

FOLKOW B and R SIVERTSSON Adaptive changes in reactivity and wall/lumen ratio in cat blood vessels exposed to prolonged transmural pressure difference Life Sci 1968 7 1283 1289

FRANK E Bestehen Beziehungen zwischen chromaffinem System und der chronishen Hypertonie des Menschen? Ein kritischer Beitrag zu der Lehre von der physio-pathologischen Bedeutung des Adrenalins Dtsch Arch klin Med 1911 103 397–412

FREIS E Hemodynamics of hypertension Physiol Rev 1960 40 27 54

FRIEDMAN S M C L FRIEDMAN and M NAKASHIMA Cationic shifts and blood pressure regulation Circulat Res 1957 5 261 267

FROHLICH E D V J KOZUL R C TARAZI and H P DUSTAN Physiological comparison of labile and essential hypertension Circulat Res 1970 26–27 Suppl I I:55–I:69

FROHLICH E D R C TARAZI H P DUSTAN and I H PAGE The paradox of β-adrenergic blockade in hypertension Circulation 1968 37 417–423

FUNDER J W J R BLAIR WEST M C CAIN K J CATT J P COGHLAN D A DENTON J F NELSON B A SCOGGINS and R D WRIGHT Circulatory and humoral changes in the reversal of renovascular hypertension in sheep by unclipping the renal artery Circulat Res 1970 27 249 258

FURUYAMA M Histometrical investigations of arteries in reference to arterial hypertension Tohoku J exp Med 1962 76 388–414

GOLDBLATT H Experimental hypertension induced by renal ischemia Bull N Y Acad Med 1938 14 523-553

GOLDBLATT H J LYNCH R F HANZAL and W W SUMMERVILLE Studies on experimental hypertension I The production of persistent elevation of systolic blood pressure by means of renal ischemia J exp Med 1934 59 347-379

GOLDRING W and H CHASIS Hypertension and hypertensive disease The Commonwealth Fund New York 1944

GREWAL R S and C L KAUL Importance of the sympathetic nervous system in the development of renal hypertension in the rat Brit J Pharmacol 1971 42 497-504

GULL W W and H G SUTTON On the pathology of the morbid state commonly called chronic Bright s disease with con tracted kidney (Arterio-capillary fibrosis) Trans of the Royal Med and Chir Soc 1872 55 273-326

GUYTON A C and T G COLEMAN Quantitative analysis of the pathophysiology of hypertension Circulat Res 1969 24 Suppl 1 1:1 1:19

HAEUSLER G and L FINCH Vascular resistance and reactivity to various vasoconstrictor agents in hypertensive rats Spontaneous Hypertension Ed by K Okamoto Igaku Shoin Ltd Tokyo Springer Verlag,Berlin-Heidelberg-New York 1972 97 102

HALLBACK M and B FOLKOW Cardiovascular responses to acute mental stress in spontaneously hypertensive rats Acta physiol scand 1974 In press

HALLBACK M Y LUNDGREN and L WEISS Reactivity to nor adrenaline of aortic strips and portal veins from spontaneously hypertensive and normotensive rats Acta physiol scand 1971 81 176-181

HALLBÄCK M Y LUNDGREN and L WEISS The distensibility of the resistance vessels in spontaneously hypertensive rats (SHR) as compared with normotensive control rats (NCR) Acta physiol scand 1974 90 57-68

HENRY J P J P MEEHAN and P.M STEPHENS The use of psychosocial stimuli to induce prolonged systolic hypertension in mice Psychosom Med 1967 29 408-432

HERD J.A W H MORSE R T KELLEHER and L G JONES Arterial hypertension in the squirrel monkey during behavioral experiments Amer J Physiol 1969 217 24-29

HINES E.A and G E BROWN A standard test for measuring the variability of blood pressure: its significance as an index of the prehypertensive state Ann Intern Med 1933 7 209-217

HINMAN A T B T ENGEL and A F BICKFORD Portable blood pressure recorder Accuracy and preliminary use in evaluating intradaily variations in pressure Amer Heart J 1962 63 663-668

HUCHARD H Maladies du coeur et des vaisseaux Paris: Doin 1889

JOHANSSON B Determinants of vascular reactivity Fed Proc 1974 In press

JOHANSSON B B LJUNG T MALMFORS and L OLSON Prejunctional supersensitivity in the rat portal vein as related to its pattern of innervation Acta physiol scand 1970 Suppl 349 5-16

JOHNSON G On certain points in the anatomy and pathology of Bright s disease of the kidney II On the influence of the minute blood-vessels upon the circulation Trans of the Royal Med and Chir Soc 1868 51 57 78

JOHNSTON C I J.S HUTCHINSON and F.A MENDELSOHN Biological significance of renin Angiotensin immunization Circulat Res 1970 26-27 Suppl II II:215-II:222

JONES A W Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats Circulat Res 1973 33 563-572

JONES T I and W.A DALE Study of peripheral autogenous vein grafts Arch Surg Chicago 1958 76 294-309

JOY M D The I tramedullary connections of the area postrema involved in the central cardiovascular response to angiotensin II Clin Sci 1971 41 89-100

JULIUS S and J CONWAY Hemodynamic studies in patients with borderline blood pressure elevation Circulation 1968 38 282 288

JULIUS S A V PASCUAL and R LONDON Role of parasympathetic inhibition in the hyperkinetic type of borderline hypertension Circulation 1971 44 413-418

JULIUS S and M.A SCHORK Borderline hypertension A critical review J chron Dis 1971 23 723-754

KALIS B L R E HARRIS M SOKOLOW and L G CARPENTER, Response to psychological stress in patients with essential hypertension Amer Heart J 1957 53 572 578

KERNOHAN J W E W ANDERSON and N M KEITH The arterioles in cases of hypertension Arch Intern Med 1929 44 395-423

LAVERTY R D D McGREGOR and E G McQUEEN Vascular reactivity in experimental hypertension New Zeald med J 1968 67 303-309

HAM J M Mechanisms in renal hypertension Proc roy Soc Med 1971 64 409-418

GHAM J M and R D COHEN Circulatory changes during the reversal of experimental hypertension Clin Sci 1962 22 69-77

LEDINGHAM J M and R D COHEN Changes in the extracellular fluid volume and cardiac output during the development of experimental renal hypertension Canad med Ass J 1964 90 292-294

LEDINGHAM J M and D PELLING Cardiac output and peripheral resistance in experimental renal hypertension Circulat Res 1967 20-21 Suppl II II:187 II:199

LIARD J F and G PETERS Mechanism of the fall in blood pressure after unclamping in rats with Goldblatt type hypertension Experientia 1970 26 743-745

LJUNG B Nervous and myogenic mechanisms in the control of a vascular neuroeffector system Acta physiol scand 1970 Suppl 349 33-68

LORIMER A R P W MACFARLANE G PROVAN T DUFFY and T D V LAWRIE Blood pressure and catecholamine responses to stress in normotensive and hypertensive subjects Cardiovasc Res 1971 5 169 173

LUND-JOHANSEN P Hemodynamics in early essential hypertension Acta med scand 1967 181 Suppl 482 1 105

MACDONALD G J., W J LOUIS V RENZINI G W BOYD and W S PEART Renal-clip hypertension in rabbits immunized against angiotensin II Circulat Res 1970 27 197 211

MAHOMED F.A Some of the clinical aspects of chronic Bright s disease Guy s Hosp Rep 1879 24 363-436

MAHOMED F.A Chronic Bright s disease without albuminuria Guy s Hosp Rep 1881 25 295-416

MELLANDER S and B JOHANSSON Control of resistance exchange and capacitance functions in the peripheral circulation Pharmacol Rev 1968 20 117 196

MENDLOWITZ M Vascular reactivity in systemic arterial hypertension Amer Heart J 1973 85 252 259

MICHELAKIS A M and R G McALLISTER The effect of chronic adrenergic receptor blockade on plasma renin activity in man J clin Endocrin 1972 34 386-394

NAGAOKA A K KIKUCHI and Y ARAMAKI Participation of tissue electrolytes and water to the spontaneous hypertension in rats Jap Circulat J 1970 34 489-497

NESTEL P J Blood-pressure and catecholamine excretion after mental stress in labile hypertension Lancet 1969 5 692-694

OKAMOTO K Spontaneous hypertension in rats Int Rev Exp Path 1969 7 227 270

OKAMOTO K Spontaneous hypertension Its pathogenesis and complications Tokyo Igaku Shoin Ltd Berlin-Heidelberg-New York Springer Verlag 1972

OKAMOTO K and K AOKI Development of a strain of spontaneously hypertensive rats Jap Circulat J 1963 27 282 293

OVERBECK H W B T SWINDALL, D F COWAN and M C FLECK Experimental renal hypertension in dogs Forelimb hemodynamics Circulat Res 1971 29 51-62

PAGE I H and J W McCUBBIN The physiology of arterial hypertension Handbook of Physiology Circulation Vol 3 1965 2163-2208

PAGE I H and J W McCUBBIN Renal hypertension Year Book Medical Publishers Inc 1968

PFEFFER M.A and E D FROHLICH Hemodynamic and myocardial function in young and old normotensive and spontaneously hypertensive rats Circulat Res 1973 32 Suppl I 1:28 1.38

PICKERING G W The role of the kidney in acute and chronic hypertension following renal artery constriction in the rabbit Clin Sci 1945 5 229 247

PICKERING G W High blood pressure London J & A Churchill Ltd 1968

PICKERING G W and M PRINZMETAL Experimental hypertension of renal origin in the rabbit Clin Sci 1938 3 357-368

ROBINSON S C and M BRUCER Body build and hypertension Arch Int Med (Chicago) 1940 66 393-417

SANNERSTEDT R Hemodynamic response to exercise in patients with arterial hypertension Acta med scand 1966 180 Suppl 458 7-83

SANNERSTEDT R Do adrenergic β-receptor blocking agents have a central nervous action? The nervous system in hypertension Ed by S Julius and M Esler Springfield Ill C C Thomas Publ 1974 in press

SELYE H C E HALL and E M ROWLEY Malignant hypertension produced by treatment with desoxycorticosteroneacetate and sodium chloride Canad med Ass J 1943 49 88 92

SHORT D S Morphology of the intestinal arterioles in chronic human hypertension Brit Heart J 1966 28 184-192

SHORT D S and A D THOMSON The arteries of the small intestine in systemic hypertension J Path Bact 1959 78 321-334

SIVERTSSON R The hemodynamic importance of structural vascular changes in essential hypertension Acta physiol scand 1970 Suppl 343 6-56

SMIRK F H and W H HALL Inherited hypertension in rats Nature 1958 182 727 728

SUWA N and T TAKAHASHI Morphological and morphometrical ana lysis of circulation in hypertension and ischemic kidney München Berlin-Wien Urban & Schwarzenberg 1971

SVEJCAR J I PREROVSKY J LINHART and J KRUML Content of collagen elastin and water in walls of the internal saphenous vein in man Circulat Res 1962 11 296-300

TIBBLIN G S -E BERGENTZ J BJURE and L WILHELMSEN Hematocrit plasma protein plasma volume and viscosity in early hypertensive disease Amer Heart J 1966 72 165-176

TOBIAN L A viewpoint concerning the enigma of hypertension Amer J Med 1972 52 595-609

TOBIAN L and J T BINION Tissue cations and water in arterial hypertension Circulation 1952 5 754-758

TOBIAN, L R OLSON and G CHESLEY Water content of arteriolar wall in renovascular hypertension Amer J Physiol 1969 216 22 24

TURNBULL, H M Alterations in arterial structure and their relation to syphilis Quart J Med 1915 8 201-254

UEDA H Y UCHIDA K UEDA T GONDARIA and S KATAYAMA Centrally mediated vaso-pressor effect of angiotensin II in man Jap Heart J 1969 10 243 247

ULRYCH M E D FROHLICH H P DUSTAN and I H PAGE Immediate hemodynamic effects of beta-adrenergic blockade with propranolol in normotensive and hypertensive man Circulation 1968 37 411-416

VAN CITTERS R L., Occlusion of lumina in small arterioles during vasoconstriction Circulat Res 1966 18 199-204

VANDONGEN, R W.S PEART and G W BOYD Adrenergic stimulation of renin secretion in the isolated perfused rat kidney Circulat Res 1973 32 290-296

VOLHARD F and T FAHR Die Brightsche Nierenkrankheit Klinik Pathologie und Atlas Springer Berlin 1914

WALLIN B G W DELIUS and K.-E HAGBARTH Comparison of sympathetic nerve activity in normotensive and hypertensive subjects Circulat Res 1973 33 9 21

WEISS L Long-term treatment with antihypertensive drugs in spontaneously hypertensive rats (SHR) Effects on blood pressure survival rate and cardiovascular design Acta physiol scand 1974 In press

WEISS L and M HALLBACK Time course and extent of structural vascular adaptation to regional hypotension in adult spontaneously hypertensive rats (SHR) Acta physiol scand 1974 In press

WEISS L Y LUNDGREN and B FOLKOW Effects of prolonged treatment with adrenergic β-receptor antagonists on blood pressure cardiovascular design and reactivity in spontaneously hypertensive rats (SHR) Acta physiol scand 1974 In press

WEITZ W Zur Ätiologie der genuinen oder vasculären Hypertension Z klin Med 1923 96 151–181

WERKÖ L and H LAGERLÖF Studies on the circulation in man IV Cardiac output and blood pressure in the right auricle right ventricle and pulmonary artery in patients with hypertensive cardiovascular disease Acta med scand 1949 133 427–436

WILSON C and F B BYROM The vicious circle in chronic Bright s disease Experimental evidence from the hypertensive rat Quart J Med 1941 10 65–93

WOLINSKY H Response of the rat aortic media to hypertension Circulat Res 1970 26 507 522

WOLINSKY H Effects of hypertension and its reversal on the thoracic aorta of male and female rats Circulat Res 1971 28 622–637

WOLINSKY H Long-term effects of hypertension on the rat aortic wall and their relation to concurrent aging changes Circulat Res 1972 30 301–309

WOOLF C M Principles of biometry New York-Chicago San Francisco D van Nostrand Co Inc 1968

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 409

ASPECTS OF THE RELATION BETWEEN FUNCTIONAL AND STRUCTURAL CARDIOVASCULAR FACTORS IN PRIMARY HYPERTENSION

Experimental studies in spontaneously hypertensive rats

BY

LILIAN WEISS

GÖTEBORG 19

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 409

From the Departement of Physiology
University of Göteborg, Sweden

ASPECTS OF THE RELATION BETWEEN FUNCTIONAL AND STRUCTURAL CARDIOVASCULAR FACTORS IN PRIMARY HYPERTENSION

Experimental studies in spontaneously hypertensive rats.

BY

LILIAN WEISS

GÖTEBORG 1974

This summary is based on studies reported in the following papers:

I The hemodynamic consequences of regional hypotension in spontaneously hypertensive and normotensive rats
B Folkow M Gurévich M Hallbäck Y Lundgren and L Weiss Acta physiol scand 1971 83 532-541

II Time course and extent of structural vascular adaptation to regional hypotension in adult spontaneously hypertensive rats (SHR)
L Weiss and M Hallbäck Acta physiol scand In press

III The effects of immunosympathectomy on blood pressure and vascular reactivity in normal and spontaneously hypertensive rats
B Folkow M Hallbäck Y Lundgren and L Weiss Acta physiol scand 1972 84 512 523

IV Effects of prolonged treatment with adrenergic β-receptor antagonists on blood pressure cardiovascular design and reactivity in spontaneously hypertensive rats (SHR)
L Weiss Y Lundgren and B Folkow Acta physiol scand In press

V Long-term treatment with antihypertensive drugs in spontaneously hypertensive rats (SHR) Effects on blood pressure survival rate and cardiovascular design
L Weiss Acta physiol scand In press

The papers are referred to in the text by their Roman numerals

CONTENTS

INTRODUCTION 5

PREVIOUS STUDIES 5

Cardiac output 5

Resistance 6
Decreased internal radius due to increased smooth muscle activity 6
Decreased internal radius due to morphological changes 9

HEMODYNAMIC CONSEQUENCES OF VASCULAR MORPHOLOGICAL CHANGES 10

Experimental background of the present studies 10

Theoretical considerations concerning the preparation and methodological approach 11

Aim of the present investigation 17

METHODOLOGICAL PRINCIPLES 19

General expe imental procedures 19
Choice of experimental model 19
Anesthesia 19
Measu ements of arterial pressure 19
The isolated hindquarter preparation 21
Statistical calculations 25

Special experimental procedures 25
Aortic ligations 25
Immunosympathectomy 26
Chronic drug treatment 26

RESULTS AND COMMENTS 27

Effects of regional hypotension 27

Immunosympathectomy 29

β-adrenergic receptor antagonists in young and od lt SHR 32

Long term hypotensive treatment in young adult and old SHR 33
1 Blood pressure after cessation of therapy 33
2 Survival rate 34
3 Effects upon the structural vascular changes 35

Effects of treatment on cardiac hypertrophy 37

GENERAL DISCUSSION 39

SUMMARY AND CONCLUSIONS 45

ACKNOWLEDGEMENTS 47

REFERENCES 48

INTRODUCTION

Despite much effort the exact background of essential (primary) hyper tension is not yet understood The first tentative description of this important disorder of regulation was presented more than 200 years ago (cf Backer 1953) However it was not until the presence of an increased intravascular pressure was demonstrated in the late 19th century (e g Mahomed 1881) that more extensive investigations were made possible The probable importance of genetical influences was suggested by Morgagni al eady in 1769 (cf Pickering 1968) but not until fairly recently have epidemiological studies clearly revealed the involvement of a polygenic inheritance (cf Pickering 1968 Miall 1971)

The present investigation was performed on a strain of rats with primary hypertension i e the spontaneously hypertensive rat (SHR; *Okamoto and Aoki 1963 Okamoto 1969) which is so far considered to be the most appropriate animal model of essential hypertension in* man Similar to human hypertension a polygenic inhe itance has been shown in these hypertensive rats Furthermore they display the same type of cardiovascular sequelloe as is seen in ma (Okamoto 1972) Interest was focused primarily on the structural cardiovascular adaptation of the systemic resistance vessels Its development and regression and its hemodynamic importance since this part of the cardiovascular system constitutes the main point of disturbance once hypertension has reached its established phase For these purposes a hemodynamic approach was used for quantitative evaluation of the vascular changes

Mean arterial pressure is determined by cardiac output and peri pheral resistance and an increased pressure may be induced by increases of one or both of these factors Furthermore a increased resistance might be due to an increased length of the vessels to an increased blood viscosity and/or to a educed cross-sectional area of the vascular bed Below these different possibilities will be briefly outlined

PREVIOUS STUDIES

Cardiac output

It has been suggested that an increased cardiac output might play an important role particularly for the initiation of essential hypertension Thus Wezler and Böger (1939) Werkö and Lagerlöf (1949) and Varnauskas (1955) observed an increased cardiac output in combination with

a largely normal peripheral resistance in hypertensive subjects These findings have been further confirmed and suggested to be present in early phases of the disease (e g Finkielman Worcel and Agrest 1965 Sannerstedt 1966 Lund-Johansen 1967 Julius and Conway 1968 Frohlich et al 1970 Julius and Schork 1971) A cardiovascular pattern associated with an increased cardiac output may indicate the presence of an increased neurogenic influence similar to that of a mild defence reaction (Eliasson et al 1951 Brod 1963 Charvat Dell and Folkow 1964 Julius Pascual and London 1971)

On the other hand there is no evidence of an increased cardiac output in the established phase of hypertension (Goldring and Chasis 1944 Freis 1960 Pickering 1968 Frohlich et al 1970) Therefore the main discussion in the literature has concerned the problem of the increased resistance at this stage of the disease

Resistance

So far no reliable studies have suggested that an increase in length of the systemic resistance vessels or a raised blood viscosity should play an important role for the well-known increase in resistance in established essential hypertension (Pickering 1968) However an increased viscosity in vitro has been reported in patients with essential hypertension (Tibblin et al 1966), but it should be pointed out that such measurements often poorly reflect the viscosity of blood in vivo (Djojosugito et al 1970)

According to the law of Poiseuille the resistance is inversely proportional to the fourth power of the internal radius of the vessel Therefore changes in the internal radius have a far greater influence on resistance to flow than changes in either viscosity or vascular length Most investigations have been concerned with the functional restrictions of the internal radius of the resistance vessels as induced by an increased vascular smooth muscle activity while the hemodynamic influence of structural vascular changes have been almost totally neglected

Decreased internal radius due to increased smooth muscle activity

Several possible mechanisms that may enhance vascular tone in hypertension are discussed in the literature 1) Increased vasoconstrictor fibre activity 2) Increased amounts of humoral vasoconstrictor agents 3) Increased smooth muscle sensitivity to vasoconstrictor influences 4) Increased myogenic tone and 5) changes in ion composition

1 Increased vasoconstrictor fibre activity to the systemic resistance vessels of hypertensive patients during rest has not been clearly demon

strated (Pickering 1936 Prinzmetal and Wilson 1936 F eis 1960 Pickering 1968 Wallin Delius and Hagbarth 1973) However more or less transient increases of sympathetic discharge to both heart and blood vessels during daily life might result in more powerful and/or frequent pressure rises in hypertensive than in normotensive subjects (Hinman Engel and Bickford 1962 Pickering 1968 Bevan Honour and Stott 1969) Such diffe encesmay at least sometimes reflect accentuated central discharge patterns to ordinary alerting situations (Hines and Brown 1933 Folkow 1960 Charvat Dell and Folkow 1964 Hall back and Folkow 1974) Recent findings of increased plasma catecholamines in patients with essential hypertension (Engelman Portnoy and Sjoerdsma 1970 DeQuattro and Chan 1972) may further support this hypothesis Furthermore it has been shown that sustained hypertension can be induced by f equently repeated direct hypothalamic stimulations (Folkow and Rubinstein 1966) or when such activations a e induced by stressful environment (e g Henry Meehan and Stephens 1967 Herd et al 1969 Gutman and Berson 1971)

2 Humoral vasoconstrictor agents may di ectly or indirectly induce hypertension and have been found in some special forms of hypertensive d'sease such as in pheocromocytoma and renal hypertension possibly also in Cushing s syndrome and Conn s disease However such agents have not been convincingly demonstrated in uncomplicated primary hypertension (Pickering 1968 Okamoto 1972)

3 Increased sensitivity (eactivity) to vasoconstrictor influences also denoted as supersensitivity or hyperreactivity is a concept which is often poorly defined in the literature (e g Sivertsson 1970) and is sometimes indiscriminately used for a whole variety of possible differen ces in vascular design and effector sensitivity It is necessary to dis tinguish between increased sensitivity or reactivity in the pharmacologi cal sense i e true smooth muscle supersensitivity and the hemodynamic aspect of vascular reactivity where also the influence of the geometri cal arrangement of the contractile elements in elation to the lumen must be considered (cf Johansson 1974 Furthermore increased eac tivity e g in the sense of an increased pressure response to a given stimulus which has been studied since the beginning of the century includes besides the possible increase in smooth muscle sensitivity and/or increased wall/lumen ratio also the complex pressure regulation of the entire cardiovascular system To exemplify a reduced responsiveness of the homeostatic baroreceptor reflexes might result in a type of hyperreactivity to pressor influences etc In general therefore the pressure esponses to cold exposure (e g Hines and Brown 1933; cf Pickering 1968 Sivertsson 1970) and the pressure and/or resistance

responses to standard doses of pressor substances (e g Clough 1920 Goldenberg et al 1948 Doyle and Black 1955 Freis 1960; cf Pickering 1968 Sivertsson 1970) have been extensively studied with out providing conclusive information about the exact background of the enhanced responses frequently observed in essential hypertension (cf Sivertsson 1970) Nevertheless it has quite often been taken almost for granted that exaggerated vascular pressor responses should primarily reflect an increased smooth muscle sensitivity (reactivity) thus neglect ing possible influences of a changed vascular structural design

In order to explore the true smooth muscle sensitivity it is advantageous to study strips of vessels though it should be realized that they do not necessarily reflect the functional characteristics of the smooth muscle of the resistance vessels which is the vascular section of interest Most studies of vascular strips have been performed on animals with various types of secondary hypertension (e g Redleaf and Tobian 1958 Mallov 1959 Gordon and Nogueira 1962) Vascular strips from rats with primary hypertension (SHR) seem to display unchanged or decreased responses to noradrenaline (Spector et al 1969 Hallbäck, Lundgren and Weiss 1971 Massingham and Shevde 1971 Shibata Kurahashi and Kuchi 1973) Ettinger Seibel and Riecher (1970) studied the reactivity to noradrenaline of isolated intact small arteries from essential hypertensive man They concluded that the arteries of hyper tensive subjects responded significantly more than those of normal subjects However quantitative hemodynamic investigations in man where the true resistance vessels rather than conduit arteries are studied (see below) are hardly in agreement with these findings (Sivertsson 1970)

4 Increased myogenic tone might theoretically be a reasonable expla nation but it has hardly been discussed in this context simply because the interest has been concentrated on extrinsic excitatory influences on the vascular effector cells (Freis 1960)

5 Changes in ion composition might lead to increased smooth muscle tone or reactivity (Friedman Friedman and Nakashima 1957 Jones 1973) *and have been suggested to be of importance for the initiation of* hypertension (Jones 1973) In the latter study performed on young spontaneously hypertensive rats the blood pressure was already considerably increased when the measurements of ionic composition and shifts were done Further investigations are needed to settle this question (Freis 1960 Nagaoka Kikuchi and Aramaki 1970)

However in the established or advanced phases such mechanisms might contribute to the inc eased resistance (Pickering 1968)

Decreased internal radius due to morphological changes

The presence of morphological changes in the systemic vascular bed of patients with high blood pressure is well known When Bright in 1836 described a state with albuminuria dropsy and hypertrophy of the heart indirect blood pressure measurements were not yet known and the morphological aspects of hypertension therefore attracted the main attention Already in 1868 Johnson reported hypertrophy of the arteriolar walls in the kidney intestine skeletal muscle pia mater and skin in Bright s disease Gull and Sutton (1872) considered the vascular changes as the primary and essential condition in hyper tension Mahomed (1881) who was the first to systematically use an indirect method for blood pressure estimations concluded that there are three stages in Bright s disease; first a functional stage where only pressure is increased second a chronic stage with organic cardiovascular and renal changes and third a terminal stage with renal failure Turnbull (1915) stated that the media hypertrophy is constantly associated with persistent elevations of blood pressure and that the degree of hypertrophy in the heart and arteries is so closely related that it is possible to form an approximate estimation of the increase in the weight of the heart from examination of the a teries Morphological measurements of media hypertrophy and wall/lumen ratio were also performed by e g Kernohan Anderson and Keith (1929) Moritz and Oldt (1937) Sommers Relman and Smithwick (1958) and Giese (1966).

Nevertheless the hemodynamic consequences of such obvious changes in the structural design of the resistance vessels were to a great deal neglected until the middle of this century One of the reasons fo this was the observation that the resistance vessels of hypertensive subjects also dilate readily in response to vasodilator agents which led to the erroneous conclusion that morphological changes were of little functional relevance Furthermore it was not possible until lately to make exact quantitative measurements of the inner radius and the wall/lumen ratio in well defined states of activity and distension of minute vessels constituting the main site of the precapillary resistance Not until it was shown that resistance was raised even during maximal dilatation and the hemodynamic consequences of such changes were considered (Folkow 1956 Folkow G imby and Thulesius 1958) was attention drawn to the functional aspects of the structural changes of the resistance vessels Thus Short and coworkers (Short and Thomson 1959 Short 1966) demonstrated by morphological measurements an increased wall/lumen ratio with reduced lumina in arterioles from patients with high blood pressure but they preferred to denote the changes as vascular contracture though the hemodynamic consequences would be the same as those outlined by Folkow (1956)

However the so far most extensive and precise morphological estimation of the wall/lumen ratio of arteries and arterioles in hypertension has been performed by Suwa s group (Furuyama 1962 Suwa and Takahashi 1971) The length of the internal elastic membrane was measured by attaching a thin thread on the magnified photomicgrograph of an arterial cross section from the histological slide and it was used as an estimate of the internal circumference The surface area of the muscula coat was then planimetrically determined allowing an estimation of the relation between internal radius and media thickness A close correlation was found between the level of blood pressure and the degree of media hypertrophy Thus the most extensive hypertrophic changes were observed in arteries and larger resistance vessels while they were gradually decreasing towards the capillary level It was therefore concluded that the major resistance to flow was offered by larger and mediumsized resistance vessels while the smallest precapillary vessels were more or less protected from the pressure rise

HEMODYNAMIC CONSEQUENCES OF VASCULAR MORPHOLOGICAL CHANGES

Experimental background of the present studies

After an initial era of morphological studies and classification the hemodynamics of essential hypertension were gradually brought into focus (cf Pickering 1968) Prinzmetal and Wilson (1936) and Pickering (1936) found by using plethysmographic methods that regional flow resistance in the forearm was higher in hypertensive than in normotensive subjects during rest as well as during dilatation secondary to nerve blockade indirect heating or arterial occlusion At that time structural changes were not considered to cause the increased resistance but that some chemical factor was maintaining a vascular hypertonus However Grant and Pearson (1938) showed that flows to the forearm, measured without previous arrest of the circulation to the hand are mixed muscle and skin flows Stead and Kunkel (1940) who were aware of this observed no difference between hypertensive and normotensive patients in blood flow of forearm hand and foot after dilatation and discussed both structural and humoral factors as background to the increased resistance in hypertension Abramson and Fierst (1942) and later Brod (1963) found an increased muscular blood flow in hypertension during rest indicating that during resting conditions the increased resistance is not uniformly distributed Brod s extensive hemodynamic explorations (1963) suggested the presence

of a specific neurohormonal discharge pattern resembling a mild defence reaction where a great proportion of the cardiac output is distributed to the skeletal muscles (Eliasson et al 1951 Abrahams Hilton and Zbrozyna 1960 Folkow and Neil 1971) In general a great number of investigations suggest that with exception for the muscles the increased resistance in established hypertension is present in most systemic circuits being unusually high in the kidneys (cf Pickering 1968)

In orde to further investigate the exact background of the in creased resistance more detailed hemodynamic analyses are required covering the entire range of vascular smooth muscle tone from the level of maximal dilatation to that of maximal constriction Such studies can provide general information not only about the hemodynamic characteristics in hypertension but also about the smooth muscle sensitivity and the design of the resistance vessels Folkow (1956) and Folkow Grimby and Thulesius (1958) demonstrated that regional flow resistance was raised even at complete relaxation of the vascular smooth muscles in essential hypertension clearly indicating a fundamental change of the structural design of the resistance vessels This finding in combi nation with the morphologically demonstrated media hypertrophy led to the theoretical deduction (Folkow 1956) that for any given increase of smooth muscle contraction the hypertensive resistance vessels would display exaggerated luminal reductions In other words the presence of an increased wall/lumen ratio should have the hemodynamic conse quences of an increased flow resistance and increased vascular reactivity without necessitating any hyperreactivity or hypersensitivity of the smooth muscle cells Conway (1963) Sivertsson and Olander (1968) and Sivertsson (1970) confirmed and extended these findings

In order to fu ther explore from the qualitative and quantitative point of view the increased wall/lumen ratio of the resistance vessels by means of a hemodynamic approach it was necessary to turn to an experimental model such as the spontaneously hypertensive rat SHR (Okamoto and Aoki 1963) allowing more invasive techniques than those possible to use in man

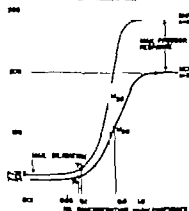
Theoretical considerations concerning the preparation and methodological approach

The quantitative hemodynamic approach to the problem of the resistance vessels i e their luminal dimensions and reactions has the great advantage that resistance to flow varies inversely to the fourth power of the average i ternal radius This marked amplification is of great importance since e g a 5 per cent reduction in the radius which is almost impossible to detect with direct morphological techniques im plies a more than 20 per cent increase in resistance to flow or in

pressure if flow is constant Furthermore by such hemodynamic measurements average values for all the parallel coupled resistance vessels under study are obtained and moreover the relative influence of the consecutive resistance sections can also be considered (Folkow et al 1974). For such reasons paired perfusion experiments (Folkow et al 1970 b) which will be further outlined in Methods were performed on isolated hindquarter preparations of spontaneously hypertensive rats (SHR) as compared to matched normal control rats (NCR) By such paired analyses both rats are exposed to almost identical experimental conditions using the same perfusate pump system drug administrations temperature etc and thus a variety of accidental disturbances are ruled out The resistance to constant flow was recorded during maximal dilatation and during graded constriction induced by noradrenaline infusions (NA) up to maximal constriction as ensured by addition of vasopressin and barium ions For an evaluation of possible differences in wall/lumen ratio and luminal reductions between SHR and NCR such a hemodynamic approach has a number of advantages over morphological measurements in addition to those mentioned above First the resistance vessels can be studied not only when their contractile elements are completely relaxed but also when they are maximally contracted against a known load and when they are kept at comparable intermediate levels of activity This allows recordings of the complete dose-response relationships in the form of resistance curves Second at known levels of smooth muscle activity the vessels can be exposed to predetermined changes in transmural pressure hence allowing precise calculations of wall distensibility from the imposed changes in pressure and flow Thi d a changed smooth muscle sensitivity to e g constrictor agents will if it is the only difference between SHR and NCR become evident as parallel shifts along the abscissa of the resistance curve and thereby allow a distinction between increased vascular reactivity secondary to increased smooth muscle sensitivity or to an increased wall/lumen ratio (Folkow et al 1970 b)

Furthermore by utilizing the constant flow technique drug concentrations will be kept constant even when adjustments in vascular dimensions take place This technique also allows measurements of the maximal contractile strength of the resistance vessels when they contract against a gradually increasing transmural pressure until an equilibrium is reached between the transmural pressure and the maximal efforts of the vessels Unless the e are any qualitative changes in the vascular walls this must closely reflect the bulk of contractile tissue in relation to the lumen

Fig 1 (from Folkow et al 1970 b) ight part illustrates the theoretically calculated resistance curves for a hypothetical hyper



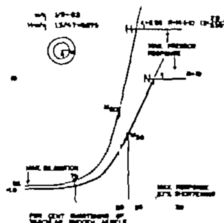


Fig 1 The left part shows the average resistance curves for SHR and NCR based on the results of 15 paired expe iments The right part shows the mathematically deduced resistance curves for two hypothetical resistance vessels H and N where H differs from N only in the respect that its media thickness is increased 30 per cent encroaching upon its lumen even at maximal dilatation Note the striking similarities between the relationships of the two sets of resistance curves with respect to 1) Resistance at maximal dilatation 2) Threshold (Th) 3) Steepness of the curves 4) 50 per cent of the maximal pressor response (M_{50}) and 5) Maximal pressor response (Folkow et al 1970 b)

tensive vessel H and a "normotensive vessel N The curves are constructed according to a number of assumptions:

1 The hypertensive vessel H has a 30 per cent thicker smooth muscle sheath than that of the normotensive vessel N

2 Outer dimensions at maximal dilatation are assumed to be the same

for the two vessels

3 The ratio between wall thickness and internal radius of the normal vessel N is set at 1:5 at maximal dilatation for normally distended completely relaxed arterioles (van Citters 1966) and this ratio will be 1 3:4 7 for the hypertensive vessel H according to assumption 1 This increased wall/lumen ratio of the hypertensive vessel H has according to the law of Poiseuille the consequence that the resistance at maximal dilatation is 28 per cent higher in H than in N The thicker wall also implies a greater stiffness

4 The cross-sectional area of the vessel wall is considered to be constant when the vessels constrict which is reasonable in the acute situation and is also supported by direct measurements on normal vessels (Baez 1969)

5 It is further assumed that constriction is normally initiated from the outermost muscle sheath which is true for contractions initiated by vasoconstrictor fibres since thei neuroeffector junctions make contact only with the adventitial muscle surface (cf Ljung 1970) This assumption seems justifiable also when the resistance vessels are exposed to exogenous noradrenaline (NA), since a recent study of the portal vein of the rat (Johansson et al 1970) suggests that the great majority of the alpha receptors are located in or close to the neuroeffector junctions For the resistance vessels this would imply that an adrenergically induced constriction displaces the inner tissue layers towards the lumen exaggerating the luminal eduction for a given smooth muscle shortening largely in proportion to the increase in wall/lumen ratio

The abscissa in the hypothetical diagram of Fig 1 is given as the percentage decrease of smooth muscle length in a scale derived from the relationship between the logarithm of the norad enaline dose and the smooth muscle shortening of an isolated aortic strip to make the scale comparable with the effects upon pressure (or resistance) in duced by known NA concentrations in the perfused vascular beds The relationship between NA concentration and smooth muscle shortening forms an S-shaped curve when a strip of smooth muscle is used and such a curve for aortic strips was utilized for transforming NA concentrations into actual shortenings of the smooth muscles By such a procedure the proportions between consecutive 5 per cent shortenings of the contractile elements of the hypothetical vessels H and N were made more directly comparable to actual shortenings of the vasculo smooth muscles caused by the different NA doses in the experiments (for details see Folkow et al 1970 b) It was arbitrarily assumed that N could maximally con tract at constant flow to such an extent that the perfusion pressure was 10 times that of maximal d'latation Since H had 30 per cent more of contractile wall mass it would then increase pressure 30 per cent more

and with an adjustment according to Laplace s law a 40 per cent higher maximal pressor response was obtained for H than for N In this way the enti e theoretical resistance curve could be deduced for both H and N

Experimentally obtained resistance curves (dose/response curves for noradrenaline infusions) from the level of maximal dilatation to maximal constriction can like the mentioned theoretically calculated resistance curves be described by five key points: 1) Resistance at maximal dila tation 2) NA *threshold (defined as the NA concentration required to* obtain a 25 per cent increase in resistance above that at maximal dila tation) 3) Steepness of the curve 4) M_{50} defined as the NA concentration required for eliciting 50 per cent of the maximal response (for comparisons with ED50 for conventional dose/response curves on muscle strips), 5) Maximal pressor response which is defined as the highest pressure level that can be reached with supramaximal NA concentrations and additional bolus doses of vasopressin and/or $BaCl_2$ during constant flow conditions

A comparison between the experimentally obtained resistance curves and the hypothetically deduced curves (Fig 1) shows striking similari ties with respect to the relationships between the hypertensive and the normotensive curves concerning all five key points mentioned above:

1 The flow resistance at maximal dilatation is increased to largely the same extent in H and SHR as compared to N and NCR

2 *The NA thresholds are identical for H SHR N and NCR*

3 The curves for H and SHR are proportionally steeper than for N and NCR respectively

4 M_{50} for both H and SHR are displaced to the left of N and NCR respectively despite equal NA thresholds This is in H the result of an increased wall/lumen ratio and the same explanation is therefore strongly suggested for SHR

5 The maximal pressor responses are proportionally raised for both H and SHR compared with N and NCR respectively

In other words in all respects the relationships between the characteristics of the SHR/NCR curves closely mimick those of the H/N ones even quantitatively Thus based on the assumptions given above the interpretation of these characteristics are that a) the increased resistance at maximal dilatation is an expression for the reduced luminal width of the resistance vessels in SHR b) the increased steepness of the resistance curve with the displacement to th left of M_{50} at identical NA *thresholds, is an expression for the raised wall/lumen* ratio and c) the higher maximal contractile response is an expression for the increased amount of smooth muscle tissue in the vessel wall of SHR

Concerning the influences of other types of vascular disturbances structural or functional a thickening of only noncontractile elements such as water logging (Tobian and Binion 1952 Tobian 1972) collagen elements or intimal thickening could explain all the differences except the increased maximal pressor response which calls for an increased amount of contractile tissue A concentric reduction of the luminal size of the resistance vessels with an unchanged wall/lumen ratio or a rarification of the resistance vessels could explain the increased resistance at maximal dilatation and the unchanged NA threshold but not the other characteristics of the resistance curve An increased sensitivity to the vasoconstrictor agents would cause a parallel shift to the left of the curve and would not be able to explain any of the changed hemodynamic characteristics in SHR Lastly if one assumes that morphologically unchanged smooth muscles have for some reason become stronger the increased steepness and maximal pressor response might be explained but not the raised resistance at maximal dilatation or the displaced M_{50} In fact one would have to combine several of these other hypothetical factors to explain the changed SHR curve and at the same time deny any hemodynamic influence of media thickening, for which there is by now an overwhelming documentation

The above mentioned hemodynamic characteristics seen in SHR (Folkow et al 1970 a b) and in essential hypertension in man (Sivertsson 1970) have also been confirmed by Haeusler and Finch (1972) These characteristics can thus when considered altogether only be explained by an increased wall/lumen ratio where the lumen is reduced even at maximal dilatation and where the wall thickening is at least to a la ge extent the result of an increased amount of smooth muscle tissue Such a hypertrophic change in structural design of the resistance vessels will result in a raised resistance level even at normal smooth muscle activity and also allow for an increased range of dilatation - constriction (vascular hyperreactivity) due to the increased wall/lumen ratio In this way the well known increased resistance in primary hypertension may be explained without necessitating any increased smooth muscle tone as a result of nervous bloodborne or local excitatory influences The more closely balanced the extent of the structural change is to the pressure rise the less is needed in enhanced smooth muscle activity to explain how the high pressure is maintained in hypertension The problem of the increased vascular reactivity has been studied also from other approaches Thus experiments on aortic strips and portal vein abolishing the influences of the wall/lumen ratio have shown that there is no inc ease in sensitivity to NA in SHR as compared to NCR (Hallbäck Lundgren and Weiss 1971 Shibata Kurahashi and Kuchi 1973) Furthermore it has ecently been shown that the resistance

vessels of SHR are considerably less distensible than those of NCR both during maximal dilatation and during stable levels of smooth muscle tone (Hallbäck Lundgren and Weiss 1974) These findings also strongly suggest an increased media thickness provided that no gross quality changes such as collagen etc of the wall components have also taken place A decreased distensibility of conduit arteries from hypertensive animals as well as from hypertensive man has earlier been shown (Feigl Peterson and Jones 1963 Greene et al 1966 Aars 1968)

More detailed analyses of the hindquarter vascular beds of SHR and NCR utilizing the isogravimetric technique have further confirmed the findings of hypertrophic vascular changes in SHR showing that they are solely confined to the precapillary resistance vessels The capillary section is largely unchanged and the SHR postcapillary resistance section displays if anything a slightly lower resistance at maximal dilatation and reduced NA responses compared with NCR (Folkow et al 1974)

Aim of the present investigation

The present series of investigations was designed to study aspects of the functional mechanisms behind the morphological changes of the resistance vessels and the associated changes in hemodynamics found both in hypertensive man and in SHR From studies on cat hindlimb (Folkow and Sivertsson 1968) and in renal hypertensive rats (Lundgren et al 1974 Lundgren 1974 a) it seems as if the hypertrophy of the precapillary resistance vessels could be considered as an exponent of a normal regional adaptation of the vascular bed to changes in pressure load regardless of the background of the increased pressure This by no means denies that at more severe and sustained pressure increases also degenerative and eve lesional elements will supervene as is well known from pathological studies in man as well as SHR (Pickering 1968 Okamoto 1972 Ooshima Yamori and Okamot 1972)

In order to estimate the influence of the regional transmural pressure per se on the development of the hypertrophic vascular changes I primary hypertension 3 week old SHR were subject to aortic li gation below the renal arteries i e at an age when hypertensive vascular changes are not yet discernable (paper I) To compare the i fluence of such an extensive hypotension on al eady established vascular changes similar aortic ligations were pe formed in adult SHR (paper II)

Furthermore the influence of the sympathetic nervous system as

a possible triggering mechanism by way of its pressure enhancing effect was studied by means of early immunosympathectomy (paper III) As mentioned there is no clear evidence of continuous sympathetic nerve hyperactivity in primary hypertension in man (cf Pickering 1968 Wallin Hagbarth and Delius 1973) but intermittent increases of sympathetic activity may well serve the same purpose in the form of e g centrally integrated defence reactions to environmental stimuli (cf Folkow 1960 Brod 1963 Charvat Dell and Folkow 1964 Folkow and Rubinstein 1966 Pickering 1968 Julius Pascual and London 1971) Recent findings of increased plasma catecholamines in patients with essential hypertension (Engelman Portnoy and Sjoerdsma 1970 De-Quattro and Chan 1972) may further support this hypothesis A long series of studies suggest an increased activity of the sympathetic neuro-hormonal system in SHR (cf Okamoto 1969 1972 Iriuchijima 1973) as a genetically linked predisposing influence evident also from the observation of exaggerated defence reactions to alerting stimuli (Folkow Hallbäck and Weiss 1973 Hallbäck and Folkow 1974) This type of autonomic discharge usually implies a more or less raised cardiac output a great proportion of which is distributed to the skeletal muscles A cardiovascular pattern of this general organisation is seen in "border-line hypertension in man (Brod 1963 Julius Pascual and London 1971) and also in young SHR (Pfeffer and Frohlich 1973) - Against such a background it was considered of interest to interfere with the β-mediated adrenergic influences in young prehypertensive SHR and in adult SHR with established hypertension (paper IV)

Finally blood pressure was kept at largely normotensive levels in young adult and very old SHR by prolonged treatment with hypotensive drugs used also in man The effects on cardiovascular structural changes on pressure development after cessation of therapy and on survival rate were investigated (paper V) The influences of age sex and duration of hypertension on the effects of the therapy were also evaluated

Thus the prese t series of investigations was designed to study the importance of several mainly functional influences concerning the development possible prevention and regression of high blood pressure hemodynamic characteristics and design of the resistance vessels

METHODOLOGICAL PRINCIPLES

General experimental procedures

Choice of experimental model

Throughout the present studies spontaneously hypertensive rats (SHR) of the Wistar strain have been used (Okamoto and Aoki 1963) originally obtained by selective breeding from a couple of Kyoto Wistar rats which showed a higher than average blood pressure In 1968 a small SHR colony came to this laboratory and breeding has continued locally though not consistently between brother and sister to reduce the risk of passenger" phenomena not relevant for the disorder to be studied

Genetical analyses of SHR suggest a polygenic mechanism involving a relatively small number of major genetic components (cf Okamoto 1972) A polygenic background seems to be present also in man (cf Pickering 1968 Miall 1971) and since SHR display practically all of the known characteristics of human essential hypertension these rats seem to represent the closest animal model available (Okamoto 1972) Furthermore their homogeneity and the easy control of treatment and environmental conditions th oughout a relatively short lifespan imply great advantages

Anesthesia

Nembutal® 4–5 mg/100 g body weight was used as anesthesia during the preparation of the hindquarters In studies I II and III measurements of arterial pressure were performed during Nembutal® anesthesia In studies I and II ligation of the aorta was performed under ether anesthesia which was also used during the cannulation of the caudal artery after which the rats were allowed to regain full consciousness (papers IV and V)

Measurements of arterial pressure

Indirect measurements Due to the size and consitution of the rat it is relatively difficult to get adequate measurements of the casual blood pressure and even more difficult to estimate the 24-hour variation and average Various indirect procedures are described in the literature (e g Byrom and Wilson 1938 Friedman and Freed 1969) but as Bunag McCubbi and Page (1971) recently stated it is often difficult to get a reliable correlation with direct measurements Others claim to obtain values well correlated with directly measured pressures If

meticulous care is taken to assure adequate pulsations using an appropriate sensing device (e g Pfeffer Pfeffer and Frohlich 1971)

In the present studies indirect pressure measurements were used only to follow the average effects of prolonged treatment within a group thus evaluating the adequacy of the dosage The method used was based on a colorimetric principle The whole rat (or only the tail in paper III) was thus warmed for about 10 minutes at 43-45°C and when the tail became pink it was blanched by application of a suprasystolic pressure inside a plexiglass cylinder surrounding the tail Then a cuff was inflated at the base of the tail to suprasystolic pressures the blanched tail was taken out of the cylinder and the cuff pressure was slowly decreased until the first signs of a skin flush appeared The pressure at which the skin flush appeared was defined as the arterial pressure Several modifications of this method and of the more conventional plethysmographic (Byrom and Wilson 1938) or microphonic (Friedman and Freed 1969) methods were also tried However the above described procedure fulfilled the needs also for immunosympathectomized rats in which indirect neurogenic vasodilatation in the tail is eliminated It was rather easy to handle and it was about as reliable as the other indirect methods in the hands of the same trained technician Moreover it was sooner or later in each animal always checked against direct pressure measurements (see below)

Direct measurements Blood pressure was measured under Nembutal® anesthesia both in the tail artery and in the carotid artery of the aorta-ligated rats in papers I and II and only in the tail in paper III. As observed in control measurements, Nembutal® lowers blood pressure in both normotensive and hypertensive rats with approximately 20-30 mm Hg However tracheostomy usually raises pressures again to a slightly larger extent and addition of unilateral occlusion of the carotid artery (by the cannulation) usually causes a further rise of 20-30 mm Hg Despite these shifts in pressure levels the relationship between the NCP and SHR pressures were largely maintained

The technique to measure blood pressure directly during awake conditions after the tail artery was cannulated during brief ether anesthesia (papers IV and V) proved to be the most reliable method Control experiments where local anesthesia was applied at the base of the tail to eliminate any pain or irritation showed that if the rat stayed awake for 2 3 hours during fairly familiar resting conditions pressure remained largely the same during this period of time in both hypertensive and normotensive rats This type of direct measurement can further be repeated several times if one starts from the middle of the tail (papers IV and V) and uses gradually more proximal parts for

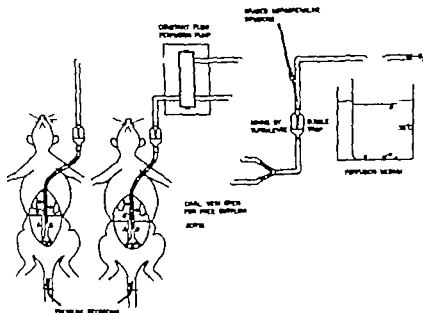


Fig 2 Schematic illustration of the experimental arrangement during paired hindquarter perfusions

cannulation

The isolated hindquarter preparation

The hemodynamic analyses were performed on paired isolated hindquarters according to earlier mentioned principles An artificial perfusion system was used employing a double Harvard pump and oxygenated Tyrode solution containing 4 per cent Ficoll® (a synthetic polymer of sucrose and epichloro-F-hydrin m w approximately 80 000; AB Pharmacia Uppsala Sweden) as plasma substitute Osmolarity was kept at approximately 300 mOsm/l and viscosity at 1 0 1 2 cP at 37°C Since the experimental series were performed in the course of several years different batches of Ficoll® with slight variations in molecular size distribution hence somewhat influencing viscosity and degree of edema formation had to be used for some of the series The temperature of the perfusate entering the rats was between 34 and 36°C

However using the paired arrangement as illustrated in Fig 2 the influence of possible changes in e g temperature viscosity osmo

larity Ficoll® and ion contents of the perfusate could be minimized Further by the paired perfusion arrangement the weight age and sex matched rats did not only get the same concentrations of constrictor substance but also the same amounts per body weight whichever the rate of infusion size of syringe or infusion apparatus used The preparation of the two rats was also performed strictly in parallel and with identical techniques

A more pure muscle preparation was used than that described originally (Folkow et al 1970 b) since measurements of both blood pressure and perfusion pressure were performed in the tail artery and therefore both the tail distal to the cannula and the feet were excluded by tight ligatures

After pressure measurement in the awake state the animals were heparinized anesthetized with Nembutal® and eviscerated after which the aortas and caval veins were freed for about 1 1 5 cm proximal to the iliac arborization The hindquarters were isolated by several standardized ligatures around all the tissues leaving the aorta and caval vein intact Then the two aortas were rapidly connected via identical polyethylene tubes to the perfusion system the caval veins were cut wide open for free outflow and perfusion was immediately started at a flow of approximately 10 ml/min x 100 g The hearts were subsequently extirpated and divided into the left and right ventricles which were weighed separately Papaverine proven to be the most effective vasodilator was given as repeated 1 mg injections in the common tube to ensure complete relaxation of the vessels However during artificial perfusion at such low flow rates the vessels gradually reach virtually complete relaxation even without dilator drugs In these respects there was never any difference between NCR and SHR thus inherent vascular tone was not better preserved in SHR

When no further relaxation could be obtained the vascular bed was considered to be in a maximally relaxed state and flow was changed randomly (5–40 ml/min x 100 g) while the pressure changes were recorded via the tail artery cannula and a transducer on the Grass Polygraph (Model 7) Flow was then set at 10 ml/min x 100 g tissue and stepwise noradrenaline (NA) infusion from subthreshold (0 04 μg/ml perfusate) to supramaximal doses (5 μg/ml perfusate) was started Finally to ensure maximal constriction supramaximal doses of vasopressin (10 IU) and $BaCl_2$ (150 mg) were added as bolus injections in the common tube Equal distribution of vasoactive agents to the two preparations was ensured by continuous shaking (by means of a motor) of the common bubble trap distal to the site of drug injection

At the end of the experiments all parts of the rat except the perfused hindquarters we e weighed and subtracted from the total body

weight in order to get an exact weight of the perfused tissues without including edema. This is necessary since edema formation occurs during NA infusion and accelerates after the administration of vasopressin and $BaCl_2$ because these drugs particularly strongly constrict the postcapillary resistance vessels (Folkow et al. 1974). Pressure flow curves during maximal dilatation and resistance curves for the resistance vessel responses were constructed and treated as described in a previous section in which the methodological approach and its advantages were considered. Below the five "key points" of the resistance curves will be discussed from the methodological point of view.

1. Resistance at maximal dilatation reflects the structurally determined average luminal width of the resistance vessels when the smooth muscles are completely relaxed. The question is how to ensure that they are completely relaxed and this was dealt with in various ways. a) By using an artificial perfusion neurogenic and circulating constrictor influences were eliminated and tissue ion composition made largely equal. This is advantageous when the aim is to study the hemodynamic consequences of a changed resistance vessel design. b) Several potent dilator substances such as nitrites and isoprenaline were tried but repeated large doses of papaverine proved to be the most effective drug. c) If sufficient amount of time was allowed to pass vascular tone diminished spontaneously probably because of factors like the low pressure and low flow rate etc. and papaverine did then not give any further relaxation even when large doses were given. d) It was furthermore controlled that pressure returned to the initial level after shortlasting increases in flow. When the vessels were not fully relaxed pressure then returned to a lower level than before the pressure rise. When a maximally dilated state was ensured in such ways there were no signs of autoregulation or other "active" changes in resistance or of any gradually increasing vascular tone.

2. The NA threshold is defined as the NA concentration required to raise resistance 25 per cent above the state of maximal dilatation which implies roughly a 5 per cent shortening of the contractile elements. The wall/lumen ratio has in this state of minimal constriction very little influence even in the fairly thickwalled resistance vessels. The greatest influence on "threshold" would then be an increased smooth muscle sensitivity displacing the resistance curve to the left even in its earliest part which was seen e.g. after immunosympathectomy (paper III).

3 Steepness of the resistance curve is expressed as the tangent of the angle between the steepest part of the curve and the abscissa and is as earlier outlined an exponent for the wall thickness in relation to the lumen during given degrees of smooth muscle shortening regardless of what has caused the wall thickening (e g muscle hypertrophy collagen elastin water endothelial thickening etc) A change only in smooth muscle sensitivity would not affect the curve steepness but rather shift the curve in a parallel manner along the abscissa When the contraction is initiated from the outer muscle layers the bulk of the muscle tissue together with all other wall elements situated inside the main line of force must be displaced towards the lumen In papers I and III correction was made for the fact that the SHR curve starts from a higher resistance tending to give a proportionally steeper angle in a coordinate system (Folkow et al 1970 b; see also Sivertsson 1970) However in the more complex studies in papers II IV and V where the main topic was to follow changes in this already known vascular characteristic of SHR no such corrections for the slopes were performed

The steepness of the resistance curve is a fairly sensitive parameter insofar as it calls for as many points of measurement as possible along the steep part of the curve and a standardized procedure for plotting the curves However the paired perfusion arrangement is here of the greatest advantage since both recordings occur simultaneously under identical conditions

4 M_{50} is defined as the concentration of NA eliciting 50 per cent of the maximal NA response This parameter is not only dependent upon the smooth muscle sensitivity but also on the wall/lumen ratio and the maximal pressor response to NA and is therefore not equivalent to ED_{50} in dose-response curves for muscle strips (Folkow et al 1970 b) M_{50} has only limited relevance in the present studies because several of the therapeutical procedures may have interfered with smooth muscle sensitivity

5 The maximal pressor response as elicited by supramaximal doses of constrictor substances such as $BaCl_2$ and vasopressin is considered to reflect the bulk of contractile elements in relation to the lumen since an increase in passive wall elements would only slightly increase this parameter (Folkow et al 1970 b) However vasopressin and $BaCl_2$ activate the postcapillary resistance vessels more strongly than NA (Folkow et al 1974) hence rapidly increasing edema formation In general edema formation was greater in NCR than in SHR tending to give NCR falsely too high maximal pressor responses because of more pronounced increases in tissue pressure However such interferences will not affect the maximal pressor responses more than about 5 per cent

as explored in other studies (Folkow et al 1974) The large doses of vasopressin and barium chloride were rather equal in potency and if vasopressin was given first the other agent added very little and vice versa

Statistical calculations

In papers I and III statistical evaluation of the hemodynamic analyses was performed according to the group comparison t test The left ventricular heart weights (papers IV and V) were also compared by means of this test

In papers II IV and V a pai ing design t test was used for sta tistical evaluation of the d'fference between the test animal and its paired control However for comparison between groups of treated SHR – NCR and untreated SHR NCR a group comparison t test of the differences was performed (Woolf 1968)

The difference between groups was considered significant at p-values below 0 05

Special experimental procedures

Aortic ligations

This procedure was performed in young prehypertensive SHR to investi gate to what an extent the development of hypertensive vascular changes are dependent upon the local transmural pressure Aortic ligation was also performed in SHR in the established phase of hypertension to follow the rate and extent of regression of the already present vascula changes The aorta was totally obstructed by a silk ligature so that the hind quarters were subsequently dependent upon collateral blood supply Al ready one day after the operation the rats seemed to be largely unaffected by the operation and postoperative mortality was low The young rats developed collaterals very rapidly and after 11 weeks hind quarter blood pressure had reached the level of 85-95 mm Hg Their gain in body weight and also that of their hindquarters was largely normal Of adult rats only females could be used presumably because of their better collateral supply via the ovaries However the adult female SHR seemed to recover almost as rapidly as young SHR and after two days they wer able to walk freely Immediately after operation local blood pressure was about 25 mm Hg and after 19 weeks about 80 mm Hg in adult SHR Ther were no signs of tissue atrophy in the hindquarters

Attempts were made to obtai a more moderat pressure reduction

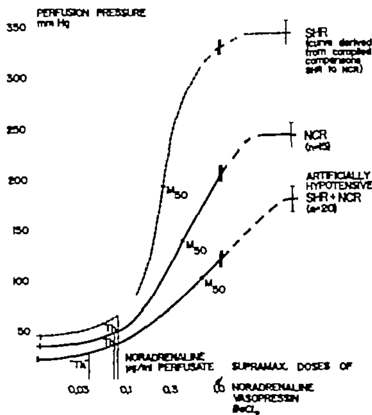


Fig 3 Average resistance curves of constant flow perfused hindquarter vascular beds from aortaligated SHR - NCR and untreated SHR - NCR Note how the three curves differ in proportion to the blood pressure in the hindquarters of aortaligated SHR NCR 97 ± 12 and 80 ± 6 mm Hg; pooled into one curve In the NCR hindquarters 145 ± 4 and in the SHR hindquarters 203 ± 8 mm Hg

maximal pressor response (0 72) but the steepness of the resistance curve (see paper II) mainly reflecting the wall/lumen ratio remained elatively increased (0 89) This may indicate that in more advanced stages of hypertension there are other elements present in the walls of the resistance vessels such as collagen and elastin which display far less regression than does the smooth muscle component (cf Wolinsky 1971 1972) Young prehypertensive SHR showed on the other hand

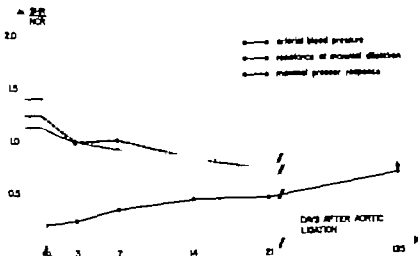


Fig 4 Diagrammatic illustration of the regional blood pressure drop and the extent of structural vascular adaptation in the hindquarters with time after aortic ligation in adult female SHR All values are expressed as the ratios for SHR/NCR and for aortaligated SHR/NCR

complete adaptation also of the steepness of the resistance curves upon pressure reduction indicating a difference in plasticity between young and adult animals in this respect

Immunosympathectomy

Immunosympathectomy was performed in newborn SHR and NCR to produce an extensive reduction at an early age of the functional trigger influence exerted by the sympathetic nervous system upon the development of high blood pressure and vascular changes At the age of 13 months when the acute experiments were performed the directly measured blood pressures were for sympathectomized SHR and NCR 139 and 113 mm Hg respectively as compared to 201 and 139 mm Hg for untreated SHR and NCR These results suggest like those of Clark (1971) that the sympatheti nervous system is of great importance for the development of primary hypertension in rats However even if the

Furthermore when the blood pressure and vascular bed of untreated NCR were compared with those of treated SHR both having similar resting blood pressures the treated SHR exhibited significantly higher values for curve steepness and maximal pressor response (Fig 6) Thus even though there was an extensive destruction of cardiovascular adrenergic nervous control in the treated SHR they were able to maintain a largely normal blood pressure However this was associated with modest hypertensive changes in resistance vessel design which might indicate that SHR have a greater tendency to mesenchymal hypertrophy to a given load This possibility has also been discussed by Folkow Grimby and Thulesius (1958) Sivertsson (1970) and Folkow et al (1973)

β-adrenergic receptor antagonists in young and adult SHR

As previously discussed the presence of a neurogenic pattern similar to a mild defence reaction has frequently been observed in early essential hypertension in man as well as in young SHR Thus in order to interfere pharmacologically with the adrenergic influences on the heart β-adrenergic receptor antagonists were administered in the drinking water (paper IV) Two and a half month old SHR still largely in the prehypertensive phase of hypertension were treated either with a β_1-receptor antagonist or with propranolol (β_1 and β_2-receptor antagonist) up to 8 months of age Adult SHR in the established" phase were treated with propranolol from 8 until 10 months of age

When treatment was interrupted at 8 months of age in the first mentioned SHR group their arterial pressure was essentially the same as when treatment was initiated being thus considerably lower than that of untreated adult SHR The hemodynamic analyses of the hind quarters showed that also the structural design of the resistance vessels was largely the same as when the therapy started i e considerably different from the situation in adult untreated SHR (Fig 7) Thus the *resistance at maximal dilatation was at a normal level and the steep*ness and the maximal pressor response were only slightly increased compared to NCR The e was no difference in these respects between the β_1-and the combined β_1 + β_2-blockers Therefore early treatment with adrenergic β receptor antagonists evidently prevented further development of the hypertensive disease but did not manage to bring the situation to an entirely normal level

Propranolol treatment in adult SHR with established hypertension did not reduce resting blood pressure although the resistance at maximal dilatation was educed as was the maximal pressor response in females

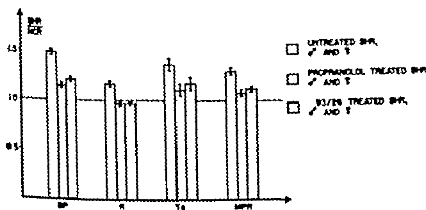


Fig 7 The ratio ± SEM between untreated (n = 26) propranolol (n = 17) or H 93/26 (n = 18) treated SHR from age 2 5 to 8 months and thei respective NCR concerning blood pressure (BP) resistance at maximal dilatation (R) steepness of the resistance curve (Tg) and maximal pressor response (MPR)

However the main exponent for the wall/lumen ratio i e the steepness of the resistance curve was not at all affected in these adult SHR

Long-term hypotensive treatment in young adult and old SHR

It was considered of interest to investigate in more detail the effects of chronic substantial pressure reduction by hypotensive drugs in young adult and old SHR concerning first the development of hypertension after cessation of therapy second the survival rate and third the effects on already established structural changes A combination of hydralazine and guanethidine was used as treatment

1 Blood pressure after cessation of therapy

SHR were treated from the age of 2 5 months when blood pressur l barely raised and when only slight vascular changes are dete table by

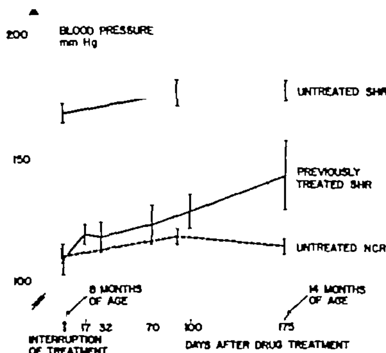


Fig 8 Changes in intraarterially measured blood pressure $\pm$ SEM of 11 SHR previously treated between 2 5 and 8 months of age as compared to 15 NCR and 15 untreated SHR and followed for 6 months after treatment was interrupted

Hemodynamic analyses The treatment was interrupted after 5 5 months when direct pressure measurements were performed During the subsequent months pressure increased quite slowly reaching 145 mm Hg only after several months (Fig 8) and was throughout far lower than in untreated SHR

2 Survival rate

Survival rate after the age of 12 months was considerably improved in SHR previously subject to treatment between 2 5 and 8 months of age as compared to untreated SHR or SHR treated only from the age of 12 months (Fig 9) There was no difference in survival rate between

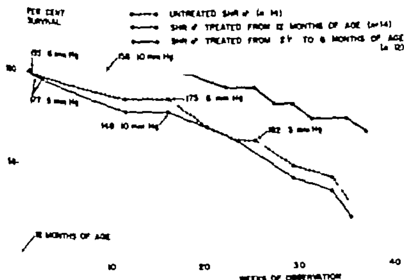


Fig 9 The survival rate of untreated male SHR of male SHR treated from 12 months of age and of male SHR previously treated between 2.5 and 8 months of age as followed between the age of 12 and 21 months Values for mean blood pressure (± SEM) ar also presented

---

rats previously treated with guanethidine-hydralazine and those previously treated with propranolol

3 Effects upon the structural vascular changes

Adult SHR - The hindquarters of SHR treated from the age of 8 months were subject to hemodynamic analyses after 5 and 10 weeks of treatment and also 2 weeks after interruption of 20 weeks of treatment The results are presented in Fig 10 as ratios between treated or untreated SHR and their normotensive controls After 5 weeks of treatment when blood pressure was normalized the resistance at maximal dilatation and the maximal contractile trength of the resistance vessels were reduced towards normal levels However the steepness of the resistance curves reflecting the wall/lume ratio emained increased No further alteration in these respects was obtained after 10 weeks of treatment However an analysis of differences between the sexes suggested a more pronounced regression of the structural vascula changes

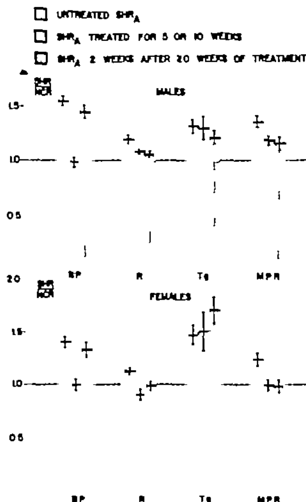


Fig 10 The ratio ± SEM between untreated (14 males and 12 females) treated (9 males and 8 females) and previously treated (7 males and 4 females) SHR and their respective NCR concerning blood pressure (BP) resistance at maximal dilatation (R) steepness of the resistance curves (Tg) and maximal pressor response (MPR)

in females than in males Similar results regarding sex differences have been reported by Wolinsky (1971 1973) on the thoracic aorta of rats earlier exposed to enal hypertension

In the rats treated for 20 weeks blood pressure was followed for two weeks after cessation of treatment During that time pressure rose fairly rapidly reaching levels only 15-20 mm Hg below those in untreated SHR controls The hindquarter vascular bed still displayed largely the same characteristics (Fig 10) as during treatment I e an unchanged increase in the steepness of the resistance curves suggesting the presence of a maintained increase in wall/lumen ratio but combined with lowered resistance at maximal dilatation and lowered maximal pressor response

Thus compared to SHR treated from young age adult SHR treated only when their hypertension was already established showed an entirely different time course in the pressure rise upon interruption of treatment Furthermore when considering the speed at which all signs of cardiovascular hypertrophy were abolished after reversal of renal hypertension in 10 week old rats (Lundgren 1974 a) it is evident that the age of the animal and the duration of the hypertensive state are both very important factors with respect to the possibilities of pressure normalization and regression of hypertensive structural changes (Wolinsky 1972)

It is probable that the signs of a remaining increase in wall/lumen ratio of the resistance vessels in adult SHR reflect the presence of enhanced amounts of collagen and other more degenerative wall changes which persist in spite of pressure reductions If an increased wall/lumen ratio is maintained it may be expected that resistance returns to supranormal levels when a normal smooth muscle tone is restored after cessation of therapy Also the bigger arteries would tend to remain stiffer (cf Wolinsky 1971 1972) which would prevent the baroreceptors from effective resetting back to normotensive pressure levels (cf Aars 1969)

Old SHR The older the organism and the higher the blood pressure the more difficult it is to normalize blood pressure and cardiovascular design This was illustrated in a group of old (12 months) severely hypertensive SHR that we e treated for 13 weeks At this age SHR display gross cardiovascular as well as renal lesions (see Okamoto 1972 Ooshima Yamori and Okamoto 1972) Treatment caused only a modest pressure reduction (to 150 mm Hg) and there was only a moderate regression in the vascular changes Particularly no significant change was seen in the parameter reflecting the wall/lumen ratio

Effects of treatment on cardiac hypertrophy

The influence on cardiac hypertrophy of the pharmacological treatments (papers IV and V) is minute in SHR This might at least I part be

characteristics as earlier shown in man (cf Folkow et al 1973) i e an increased resistance at maximal dilatation in most vascular beds and increased resistance responses to graded infusions of noradrenaline (NA) though without signs of any increased smooth muscle sensitivity to NA Furthermore the isolated SHR hindquarters displayed increased maximal pressor responses reflecting an enhanced contractile strength of the media These hemodynamic characteristics were theoretically analysed and interpreted as exponents of a changed design of the resistance vessels i e an increased wall/lumen ratio associated with narrowed lumina The increased wall thickness is at least in early phases predominantly constituted by an increased bulk of contractile tissue Recent studies (Folkow et al 1974) have revealed that this change is strictly confined to the precapillary resistance vessels

The present investigations were undertaken to analyse why when and how rapidly such changes take place in the course of hypertensive disease and how they can be either prevented or brought into regression once they are already established By aortic ligation an extensive reduction in regional pressure was produced both in young and adult SHR In young SHR the development of the characteristic vascular changes was thereby entirely prevented However in adult SHR with already established hypertension, the extensive local pressure reduction rapidly diminished the vascular alterations though less completely than in young SHR Thus signs of a partly maintained increase of *wall thickness perhaps reflecting the presence of an increased* collagen component in this later phase of hypertension was demonstrated (see also below) These results in combination with the findings in hypotensive cat hindlimb (Folkow and Sivertsson 1968) and in young renal hypertensive rats (Lundgren et al 1974 Lundgren 1974 a) where the adaptive structural changes rapidly follow the blood pressure changes in both directions suggest that both heart and resistance vessels readily respond with reversible hypertrophy to regional or general in- ...oses in pressure load whatever is the initiating mechanism behind pressure change Thus the structural changes observed in primary ...pertension seem to be a qualitatively normal adaptation to a func *...onally increased pressor load*

Neurogenic mechanisms are likely to serve as a triggering influence and early phases of primary hype tension are often characterized by a pressure rise associated with an increased cardiac output and increased flow to the skeletal muscle (e g Brod 1963 Julius Pascual and London 1971 Pfeffer and F ohlich 1973) These characteristics suggest the involvement of a mild defence reaction i e the response pattern usually elicited by a variety of alerting stimuli (Folkow and Neil 1971) Studies by Okamoto and his group provide evidence

of an enhanced neurohormonal discharge to the cardiovascular system in SHR (Okamoto 1969 Okamoto 1972 also Iriuchijima 1973) In addition alerting stimuli induce increased and more prolonged rises in pressure and heart rate in SHR than in NCR (Folkow Hallbäck and Weiss 1973 Hallbäck and Folkow 1974) Studies of patients with essential hypertension also seem to suggest such a hyperreactivity (e g Kalis et al 1957 Nestel 1969 Lorimer et al 1971)

Since such reaction patterns involve an accentuated neurogenic drive on the heart as an important component chronic treatment with β-adrenergic blockers seemed to be a suitable tool for exploring the importance of this component for the development of established hypertension However it is hardly possible at present to associate the effects of this long-term intensive treatment with any specific function mediated by β-adrenergic receptors since the drugs used display a diverse mode of action perhaps also affecting central autonomic mechanisms (e g Bühler et al 1972 Fitzgerald Wale and Austin 1972 Tarazi and Dustan 1972 Sannerstedt 1974) Whatever the dominant mode of action this type of treatment prevented further development of hypertension and its vascular sequellae when used in young prehypertensive SHR but in the adult SHR the high blood pressure was not at all influenced These findings suggest that the β-receptor antagonists used have somehow critically interfered with excitatory influences on the cardiovascular system in young SHR thereby also preventing the structural vascular changes which appear to be crucial for the development of established primary hypertension On the other hand preventive propranolol treatment of this type did not have any effect on the development of renal hypertension suggesting that the trigger mechanisms in renal hypertension are of a different nature (Lundgren 1974 b)

The importance of the sympathetic nervous system for the development of primary hypertension was further confirmed by studies on immunosympathectomy in newborn SHR causing an extensive reduction of adrenergic excitatory i fluences in general This treatment also prevented the development of hypertension although the treated SHR remained at a higher pressur level tha similarly treated NCR Despite the fact that the treated SHR displayed the same blood pressure as untreated NCR there were still significant tructural changes in thei vascular beds Possibly these changes reflect that SHR are genetically somewhat more prone to respond to a give pressure load with cardiovascular hypertrophy The considerable cardiac hypertrophy seen in SHR treated with β-receptor a tagonists from an early age may point in this direction I this connection it i of interest that a prevalence for a genetically determined mesomorphic body build

has been observed in human essential hypertension (Robinson and Brucer 1940) This finding probably implies that the tissues in general including the cardiovascular system tend to respond somewhat more than normally with hypertrophy when exposed to an increased functional load (Folkow 1956 Folkow Grimby and Thulesius 1958 Folkow et al 1973) Comparisons between renal hypertensive rats with long-standing hypertension and SHR may also point in this direction (Folkow et al 1973 Lundgren et al 1974)

The procedures which brought SHR closest to similarly treated NCR with respect to blood pressure was the treatment with guanethidine and hydralazine i e a general peripheral sympathetic blockade in association with a depression of precapillary myogenic tone Also in adult SHR with not too far advanced hypertensive changes this type of treatment caused efficent pressure reduction and considerable regression of the changes of the resistance vessels in a relatively short period of time Even in advanced hypertension some reduction in pressure and alteration of the structural vascular changes could be obtained However in both these phases of hypertension the increased left ventricular weight as well as the increased wall/lumen ratio of the resistance vessels seemed to remain largely unchanged These findings probably reflect the presence of structural changes involving e g collagen invasion which is far more persistent than uncomplicated muscle hypertrophy Several investigations mainly dealing with larger arteries suggest that along with muscle hypertrophy there is also an increase of fibrous tissue elements which may develop more slowly but may also be less reversible (Rodbard 1970 Wolinsky 1970 1971 1972) According to Olsen (1971) development of fibrous elements in the vascular walls can be traced as early as within a few days after acute hypertension Long duration of the hypertensive state thus tends to introduce more or less irreversible structural changes in the cardiovascular system as well as gross lesions in e g kidneys (Okamoto 1969) which in turn may cause an additional element of renovascular hypertension Once such more persistent changes in the resistance vessel wall occur it is far more difficult to normalize the cardiovascular situation This is to be compared with the total egression of the vascular changes seen after reversal of shortlasting renal hypertension in young rats (Lundgren 1974 a)

Sex also seems to be of importance in these respects since the females were more responsive to treatment than the males This is in agreement with the findings of Wolinsky (1972 1973) suggesting a depressive influence of female sex hormones upon the development of collagen and elastin in the aorta during renal hypertension

When treatment was i terrupted in SHR exposed to preventive

treatment from youth into adult age blood pressure rose quite slowly contrary to the situation when treatment had been started only in adult age In the latter case blood pressure increased rapidly upon cessation of treatment These findings suggest that the response of the tissue to the functional load is more vivid and efficient at an early age since the development of hypertension is much slower and less severe if the neurogenic impact is delayed until adult age Freis et al (1972) made similar observations on SHR concerning development of blood pressure and vascular lesions after cessation of prolonged pharmacological treatment

In the present study it seems possible to obtain an evaluation of the influence of connective tissue formation in the resistance vessels on the course of hypertension and on the possibilities to induce effective regression of the disease by therapy The maintained increase in wall/lumen ratio in adult treated SHR might explain why it is difficult to maintain a lowered blood pressure if treatment is interrupted If the wall/lumen ratio remains increased a return of normal smooth muscle tone after cessation of therapy would tend to again result in a raised resistance Furthermore changes in wall structure maintaining an increased wall stiffness at the site of the baroreceptors would interfere with their resetting to a lower level during the treatment (cf Aars 1969) Once substantial collagen invasion has occurred with in the cardiovascular high-pressure sections the homeostatic reflex control would tend to restore the hypertensive situation, despite the therapeutic efforts

Summarizing these considerations it ca be stated first and foremost that the changes in cardiovascular design are secondary to functional increases in average pressure whether intermittent or continuous and whichever thei background This by no means denies that the extent of these changes for a given pressure increase can be moderated by other influences If the vascular bed can be protected from such a pressure load from young age these changes are largely prevented On the other hand the older the individual gets and the longer the changes have been present the more difficult it is to induce thei regression because the early smooth muscle hypertrophy probably becomes complicated by slower but more persistent connective tissue changes and degenerative phenomena Females are in a better situation than males in these respects

If hypotensive treatment is given from young age over a considerable period of time and is thereafter interrupted there will only be a quite slow and modest progression of blood pressure over a long period of time Furthermore such a period of early treatment considerably increases survival rate after 12 months of age even when

compared with SHR which are under permanent treatment but only from this adult age It therefore seems evident from these studies that for any kind of hypertension the time in life when the functional excitatory influences are changed is crucial for the further development of the disease The tissue response to the functional pressure load seems to be greatest at a young age whereas in older individuals the tissues react more slowly Therefore the morphological cardiovascular adjustments which are so important for the establishment and maintenance of a truly hypertensive state are likely to develop more slowly with age

The inherent hyperreactivity concerning qualitatively normal autonomic mechanisms conveying neurogenic pressor responses to alerting stimuli seen in SHR and perhaps also present in essential hypertension in man, might serve as an efficient trigger mechanism Further since sustained increases in blood pressure can be induced by topic hypothalamic stimulation (Folkow and Rubinstein 1966) and by stressful environment (Henry Meehan and Stephens 1967 Gutman and Benson 1971) even in otherwise normotensive individuals it is evident that exogenous influences may further enhance the functional load upon the cardiovascular system and thus be of considerable importance for the development of an established hypertensive state Structural adaptation in response to the increased functional load gradually occurs and finally in the established phase it may to a great extent be responsible for the increased resistance and blood pressure However it might also be so that other genetically linked predisposing elements than those of neurogenic origin have to contribute in case a more considerable and persistent pressure rise should be established One such additional element in primary hypertension may simply be a somewhat enhanced tendency of mesenchymal tissues to respond with hypertrophy when exposed to increased load If so primary hypertension might be characterized by quantitative rather than qualitative differences from the normal individual with respect to both these elements i e a increased central neurogenic reactivity and and increased tendency towards tissue hypertrophy

SUMMARY AND CONCLUSIONS

The present series of experiments was performed to explore the relation ships between functional and structural factors for the establishment of the raised resistance in primary hypertension as studied in spontaneously hypertensive rats (SHR) by means of a quantitative hemodynamic approach Earlier hemodynamic studies in man and SHR have revealed an increase in wall thickness in the systemic resistance vessels at least in early phases mainly consisting of smooth muscle hypertrophy with encroachment upon the lumen in most vascular circuits even during maximal dilatation

1 Regional hypotension was performed by means of aortic ligature I young prehypertensive SHR and in adult SHR with established hyper tension as well as in matched normotensive control rats (NCR) This procedure prevented respectively reduced the structural changes characteristic of hypertensive resistance vessels It was concluded that an increased transmural pressure is the main prerequisite for the develop ment and maintenance of these structural vascular changes and that they are exponents of a per se normal adaptation in design present also in normotensive vascular beds

2 Interference with the sympathetic influences upon the cardiovascular system in SHR and NCR was performed by means of a) adrenergic β-receptor antagonists and b) Immunosympathectomy
a) Intensive treatment with adrenergic β-receptor antagonists in young SHR largely prevented both the further development of hypertension and the structural cardiovascular changes However similar treatment of adult SHR did not lower the already raised blood pressure and had only minor effects on the cardiovascular changes It was concluded that interferences with sympathetic effects mediated by peripheral and per haps also central β-receptor mechanisms can prevent the further de velopment of hypertension in young animals but can only to a minor degree affect already established hypertension I adult SHR
b) Immunosympathectomy at birth prevented the development of high blood pressure in SHR thus also indicating an important role of the sympathetic nervous ystem for the initiation of this type of primary hypertension I rats However despit the extensive cardiovascular de nervation SHR were abl to maintai their blood pressure at a normal level although it was higher than in similarly treated NCR In addition the treated SHR displayed some igns of hypertensive vascular changes compared with untreated NCR but to a educed extent than in untreated SHR These results might lso indicate that SHR for a given pressure

load display a somewhat increased tendency of cardiovascular hyper trophy compared with NCR

3 The effects of chronic substantial pressure reduction by means of treatment with guanethidine-hydralazine in young adult and old SHR were investigated concerning blood pressure after cessation of therapy *survival rate and structural cardiovascular changes*
a) It was shown that after cessation in the adult age of treatment which had kept blood pressure at a normal level from early age there was only a slow and modest blood pressure rise over several months Furthermore the survival rate was considerably improved by such an early period of hypotensive treatment compared with untreated SHR or with SHR treated only from adult age There was no difference in these respects between rats treated from young age with guanethidine-hydralazine and those treated from young age with propranolol
b) Prolonged guanethidine-hydralazine treatment of adult SHR keeping them at normotensive levels for long periods did not result in full re gression of the vascular changes leaving obvious signs of an increased wall/lumen ratio of the resistance vessels This might reflect the addition of more permanent wall changes in long standing hypertension as a result of e g collagen invasion Probably as a result of such preserved vascular changes in adult SHR blood pressure rapidly returned to levels rather close to those of untreated SHR upon cessation of therapy Comparing the sexes regression of the vascular changes tended to be more extensive in the females

It was concluded that central neuro-hormonal excitatory mecha nisms whether inherent or exogenous in origin seem to be crucial for the initiation of the primary hypertension in SHR and that the cardiovascular structural alterations represent secondary adaptive changes However these changes seem to be so rapidly developed that they become in time more or less intertwined with the functional excitatory influences Furthermore it is not impossible that the structural cardiovascular adaptation to a given pressure load may be somewhat more pronounced in extent in SHR Other general influences such as age sex and duration of the high pressure state are also important both for the establishment of hypertension and for the possibilities of its reversion

ACKNOWLEDGEMENT

I wish to express my deep gratitude to my teacher Professor Björn Folkow under whose guidance it has been my privilege to work His advice and help have been of the greatest value to me during the performance of this work

I also wish to thank Margareta Hallbäck Yen Lundgren and Moisie Gurévich for very stimulating collaboration

For all skilful technical assistance I wish to thank Gunnel Andersson and Ulla Axelsson Thanks are also due to Kerstin Lundgren and Sylvia Palmquist for typing the manuscripts to Thomas Palm for the many photographs and to Gösta Magnusson for help in maintaining a breeding stock of SHR

The valuable help of Dorothy Stage for scrutinizing the English text in this summary is gratefully acknowledged

This investigation was supported by grants from the Swedish Medical Research Council (No 14X 16) from the Faculty of Medicine University of Göteborg from Svenska Sällskapet för Medicinsk Forskning and from the Swedish National Association against Heart and Chest Diseases

AB Hässle Mölndal Sweden generously covered part of the expenses for a technician and also supplied us with H 93/26 Thanks are also due to Ciba-Geigy Basel Switzerland for generous gifts of Apresolin ® Ecolid ® and Ismelin ® to ICI Ltd Great Britain for generous supply of propranolol to Pharmacia Uppsala Sweden for gifts of Ficoll du ing the initial experimental series and to Wellcome Reagents Ltd Beckenham Kent England for nerve growth factor antiserum

FEIGL E O L H PETERSON and A W JONES Mechanical and chemical properties of arteries in experimental hypertension J clin Invest 1963 42 1640-1647

FERGUSON D J and R L VARCO The relation of blood pressure and flow to the development and regression of experimentally induced pulmonary arteriosclerosis Circulat Res 1955 3 152-158

FINKIELMAN S M WORCEL and A AGREST Hemodynamic patterns in essential hypertension Circulation 1965 31 356-368

FITZGERALD J D J L WALE and M AUSTIN The haemodynamic effects of (+)-propranolol dexpropranolol oxprenolol practolol and sotalol in anaesthetized dogs Europ J Pharmacol 1972 17 123-134

FOLKOW B Structural myogenic humoral and nervous factors controlling peripheral resistance In Hypotensive Drugs Proc of a symposium on hypotensive drugs and the control of vascular tone in hypertension Ed by M Harington London Pergamon Press 1956 pp 163 174

FOLKOW B Haemodynamic responses to cortical and hypothalamic stimulation In Czech Cardiol Soc Proc Joint WHO-symposium on Pathogenesis of Essential Hypertension Prague, State Medical Publ House 1960 pp 247-255

FOLKOW B G GRIMBY and O THULESIUS Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta physiol scand 1958 44 255-272

FOLKOW B M HALLBACK Y LUNDGREN R SIVERTSSON and L WEISS Importance of adaptive changes in vascular design for establishment of primary hypertension studied in man and in spontaneously hypertensive rats Circulat Res 1973 32 33 Suppl I I:2 I:13

FOLKOW B M HALLBÄCK Y LUNDGREN and L WEISS Structurally based increase of flow resistance in spontaneously hypertensive rats Acta physiol scand 1970 a 79 373 378

FOLKOW B M HALLBÄCK Y LUNDGREN and L WEISS Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats Acta physiol scand 1970 b 80 93 106

FOLKOW B M HALLBÄCK and L WEISS Cardiovascular responses to acute mental stress in spontaneously hypertensive rats Clin Sci 1973 45 131s 133s

FOLKOW B M HALLBACK Y LUNDGREN L WEISS I ALBRECHT and S JULIUS Analysis of design and reactivity" of series coupled vascular sections in spontaneously hypertensive rats (SHR) Acta physiol scand In press

FOLKOW B and E NEIL Circulation London-New York-Toronto Oxford University Press Inc 1971

FOLKOW B and E H RUBINSTEIN Cardiovascular effects of acute and chronic stimulations of the hypothalamic defence area in the rat Acta physiol scand 1966 68 48 57

FOLKOW B and R SIVERTSSON Adaptive changes in reactivity and wall/lumen ratio in cat blood vessels exposed to prolonged transmural pressure difference Life Sci 1968 7 1283 1289

FREIS E D Hemodynamics of hypertension Physiol Rev 1960 40 27 54

FREIS E D D RAGAN H PILLSBURG III and M MATHEWS Alteration of the course of hypertension in the spontaneously hypertensive rat Circulat Res 1972 31 1 7

FRIEDMAN M and S C FREED Microphonic manometer for indirect determination of systolic blood pressure in the rat Proc of the Society for Experimental Biology and Medicine 1949 70 670-672

FRIEDMAN S.M C L FRIEDMAN and M NAKASHIMA Cationic shifts and blood pressure regulation Circulat Res 1957 5 261 267

FROHLICH E D V J KOZUL R C TARAZI and H P DUSTAN Physiological comparison of labile and essential hypertension Circulat Res 1970 26-27 Suppl 1 1:55-1:69

FURUYAMA M Histometrical investigations of arteries in reference to arterial hypertension Tohoku J Exp Med 1962 76 388-414

GIESE J The pathogenesis of hypertensive vascular disease (Thesis) Copenhagen Munksgaard 1966

GOLDENBERG M K L PINES E de F BALDWIN D G GREENE and C E ROH The hemodynamic response of man to nor-epinephrine and epinephrine and its relation to the problem of hypertension Am J Med 1948 5 792-806

Response to psychological stress in patients with essential hypertension Amer Heart J 1957 53 572 578

KERNOHAN J W E W ANDERSON and N M KEITH The arterioles in cases of hypertension Arch Intern Med 1929 44 395–423

LAVERTY R D D McGREGOR and E G McQUEEN Vascular reactivity in experimental hypertension New Zeald med J 1968 67 303 309

LIEBOW A A Situations which lead to changes in vascular patterns In Handbook of Physiology Circulation Vol 2 Chapt 37 Ed W F Hamilton and R Dow Washington American Physiological Society 1963 pp 1251-1276

LJUNG B Nervous and myogenic mechanisms in the control of a vascular neuroeffector system Acta physiol scand 1970 Suppl 349 33–68

LORIMER A R P W MACFARLANE G PROVAN T DUFFY and T D V LAWRIE Blood pressure and catecholamine responses to stress in normotensive and hypertensive subjects Cardiovasc Res 1971 5 169–173

LUNDGREN Y Regression of structural cardiovascular changes after reversal of experimental renal hypertension in rats Acta physiol scand 1974a In press

LUNDGREN Y Blood pressure and vascular design in renal hypertension in rats after prolonged propranolol treatment Acta physiol scand 1974b In press

LUNDGREN Y M HALLBACK L WEISS and B FOLKOW Rate and extent of adaptive cardiovascular changes in rats during experimental renal hypertension Acta physiol scand In press

LUND JOHANSEN P Hemodynamics in early essential hypertension Acta med scand 1967 181 Suppl 482

MAHOMED F A Chronic Bright s disease without albuminuria Guy s Hosp Rep 3rd ser 1881 25 295–416

MALLOV S Comparative eactivities of aortic strips from hypertensive and normotensive rats to epinephrine and levarterenol Circulat Res 1959 7 196-201

MASSINGHAM R and S SHEVDE Aortic reactivity and electro-

physiology in normotensive rats spontaneously hypertensive rats and rats made hypertensive with desoxycorticosterone plus salt Brit J Pharmacol 1971 43 868-870

MENDLOWITZ M Vascular reactivity in systemic arterial hyper tension Amer Heart J 1973 85 252 259

MIALL W E Heredity and hypertension Practitioner 1971 207 20-27

MORITZ A R and M R OLDT Arteriolar sclerosis in hypertensive and non-hypertensive individuals Amer J Path 1937 13 679 728

NAGAOKA A K KIKUCHI and Y ARAMAKI Participation of tissue electrolytes and water to the spontaneous hypertension in rats Jap Circulat J 1970 34 489-497

NESTEL P J Blood-pressure and catecholamine excretion after mental stress in labile hypertension Lancet 1969 692-694

OKAMOTO K Spontaneous hypertension in rats Int Rev Exp Path 1969 7 227 270

OKAMOTO K Spontaneous Hypertension Tokyo Igaku Shoin Ltd 1972

OKAMOTO K and K AOKI Development of a strain of spontaneously hypertensive rats Jap Circulat J 1963 27 282 293

OLSEN F Correlation between infiltration of mononuclear cells and production of connective tissue in acute hypertensive vascular disease Acta path microbiol scand 1971 Section A 79 15-21

OOSHIMA A Y YAMORI and K OKAMOTO Cardiovascular lesions in the selectively-bred group of spontaneously hypertensive rats with severe hypertension Jap Circulat J 1972 36 797-812

PFEFFER M A and E D FROHLICH Hemodynamic and myocardial function in young and old normotensive and spontaneously hypertensive rats Ci culat Res 1973 32 33 Suppl I I:28-I 35

PFEFFER J M M A PFEFFER and E D FROHLICH Validity of an indirect tail-cuff method for determining systolic arterial pressure in unanesthetized normotensive and spontaneously hypertensive rats J Lab cli Med 1971 78 957 962

PICKERING G W The peripheral resistance in persistent arterial

hypertension Clin Sci 1936 2 209 235

PICKERING G High blood pressure London J & A Churchill Ltd 1968

PRINZMETAL M and C WILSON The nature of the peripheral resistance in arterial hypertension with special reference to the vasomotor system J clin Invest 1936 15 63-83

REDLEAF P D and L TOBIAN The question of vascular hyper responsiveness in hypertension Circulat Res 1958 6 185-193

ROBINSON S C and M BRUCER Body build and hypertension Arch Intern Med 1940 66 393-417

RODBARD S Negative feedback mechanisms in the architecture and function of the connective and cardiovascular tissues Perspect Biol Med 1970 13 507-527

SANNERSTEDT R Hemodynamic response to exercise in patients with arterial hypertension Acta med scand 1966 180 Suppl 458

SANNERSTEDT R Do adrenergic β-receptor blocking agents have a central nervous action? In The nervous system in hypertension Ed by S Julius and M Esler Springfield Illinois C C Thomas Publishers To be published

SEN S R TARAZI P KHAIRALLAH and F M BUMPUS Biochemical correlates in cardiac hypertrophy in spontaneously hypertensive rats Circulation 1973 48 Suppl IV IV-46

SHIBATA S KURAHASHI and M KUCHII A possible etiology of contractility impairment of vascular smooth muscle from spontaneously hypertensive rats J Pharmacol exp Therap 1973 185 406-417

SHORT D Morphology of the intestinal arterioles in chronic human hypertension Brit Heart J 1966 28 184 192

SHORT D S and A D THOMSON The arteries of the small intestine in systemic hypertension J Path Bact 1959 78 321 334

SIVERTSSON R The haemodynamic importance of structural vascular changes in essential hypertension Acta physiol scand 1970 Suppl 343

SIVERTSSON R and R OLANDER Aspects of the nature of the increased vascular resistance and increased "reactivity to nor

adrenaline in hypertensive subjects Life Sci 1968 7 1291 1297

SOMMERS S C A S RELMAN and R H SMITHWICK Histologic studies of kidney biopsy specimens from patients with hyper tension Amer, J Path 1958 34 685-715

SPECTOR S J H FLEISCH H M MALING and B B BRODIE Vascular smooth muscle reactivity in normotensive and hypertensive rats Science 1969 166 1300-1301

STEAD E A Jr and P KUNKEL Nature of peripheral resistance in arterial hypertension J clin Invest 1940 19 25-33

SUWA N and T TAKAHASI Morphological and morphometrical analysis of circulation in hypertension and ischemic kidney München-Berlin-Wien Urban & Schwarzenberg 1971

ŠVEJCAR J I PŘEROVSKY J LINHART and J KRUML Content of collagen elastin and water in walls of the internal saphenous vein in man Circulat Res 1962 11 296-300

TARAZI R C and H P DUSTAN Beta-adrenergic blockade in hyper tension Amer J Cardiol 1972 29 633-640

TIBBLIN G S E BERGENTZ J BJURE and L WILHELMSEN Hematocrit plasma protein plasma volume and viscosity in early hypertensive disease Amer, Heart J, 1966 72 165-176

TOBIAN L A viewpoint concerning the enigma of hypertension Amer J Med 1972 52 595-609

TOBIAN L and J T BINION Tissue cations and water in arterial hypertension Circulation 1952 5 754 758

TURNBULL H M Alterations in arterial structure, and their relation to syphilis Quart J Med 1915 8 201 254

WALLIN B G W DELIUS and K E HAGBARTH Comparison of sympathetic nerve activity in normotensive and hypertensive subjects Circulat Res 1973 33 9 21;

VanCITTERS R L Occlusion of lumina in small arterioles during vasoconstriction Circulat Res 1966 18 199-204

VARNAUSKAS E Studies i hypertensive cardiovascular disease with special reference to cardiac function Scand J cli Lab Invest 1955 7 Suppl 17

WERKÖ L and H LAGERLÖF Studies on the circulation in man IV Cardiac output and blood pressure in the right auricle right ventricle and pulmonary artery in patients with hypertensive cardiovascular disease Acta med scand 1949 133 427-436

WEZLER K and A BÖGER Die Dynamik des arteriellen Systems Der arterielle Blutdruck und seine Komponenten Ergebn Physiol 1939 41 292-606

WOLINSKY H Response of the rat aortic media to hypertension Circulat Res 1970 26 507 522

WOLINSKY H Effects of hypertension and its reversal on the thoracic aorta of male and female rats Circulat Res 1971 28 622 637

WOLINSKY H Long-term effects of hypertension on the rat aortic wall and their relation to concurrent aging changes Circulat Res 1972 30 301 309

WOLINSKY H Mesenchymal response of the blood vessel wall A potential avenue for understanding and treating atherosclerosis Circulat Res 1973 32 543-549

WOOLF C M Principles of biometry New York Chicago San Francisco D van Nostrand Company Inc 1968

ZAIMIS E Immunological Sympathectomy Scientific Basis of Med Annual Rev 1967 59 73

ZAIMIS E The usefulness of the immunosympathectomized animal in pharmacological studies In Nerve Growth Factor and its Antiserum Ed by E Zaimis London Athlone Press 1972

ACTA PHYSIOLO[illegible] [illegible]
[illegible]

RELATION BETWEEN [illegible]
MYELIN LAMELLAE AND [illegible]
IN FIBRES OF VENTRAL [illegible]
AND OPTIC NERVE IN [illegible]
AND REHAB[illegible]

An ultra[illegible]

[illegible]

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 410

From the Neuropathological Laboratory Institute
of Pathology University of Göteborg, Sweden

RELATION BETWEEN THE NUMBER OF MYELIN LAMELLAE AND AXON CIRCUMFERENCE IN FIBRES OF VENTRAL AND DORSAL ROOTS AND OPTIC NERVE IN NORMAL, UNDERNOURISHED AND REHABILITATED RATS

An ultrastructural morphometric study

by

ANDERS SIMA

Printed in Sweden, Gotab Kungälv 1974

CONTENTS

I	Introduction	5
II	Material and methods	7
	Animals	7
	Animal environment	7
	Diet	7
	Undernutrition	7
	Nutritional rehabilitation	8
	Histological technique	8
	Counting techniques and presentation of the material	8
	Statistical methods	9
III	Results	11
	Ventral root	11
	Dorsal root	14
	Optic nerve	14
IV	Discussion	22
V	Summary	25
VI	References	26
VII	Tables	28

I INTRODUCTION

Nutritional deprivation initiated early in life may have deleterious effects on both the central and peripheral nervous system (Bass et al 1970 Clos and Legrand 1969 1970 Dickerson et al., 1967 Dickerson and Walmsley 1967 Dobbing, 1964 Hedley Whyte 1973 Sima, 1974) Nutritional rehabilitation following periods of undernutrition has revealed irreversible distortions of central and peripheral nervous system, depending on the onset and severity of undernutrition and the onset of refeeding (Dobbing, 1968 1971 Hedley Whyte 1973 Sima 1974 Sima and Sourander 1974) Previous studies have shown that protein-calorie undernutrition instituted early in life causes retardation in nerve fibre growth of both central and peripheral nerve tracts of the rat (Clos and Legrand 1969 1970 Hedley Whyte and Meuser 1971 Hedley Whyte 1973 Sima et al. 1971 Sima, 1974 Sima and Sourander 1974) As demonstrated by Hedley Whyte and Meuser (1971) both axonal growth and deposition of myelin are affected to the same extent in rat sciatic nerve by undernutrition Recent examinations by Hedley Whyte (1973) have, however indicated that in the rat sciatic nerve the deposition of myelin is more affected by undernutrition than the axonal growth. Findings from our laboratory have revealed irreversible impairments in total nerve fibre calibre growth in the sensory dorsal root (Sima, 1974) and in the optic nerve (Sima and Sourander 1974) of prenatally undernourished rats, subsequently nutritionally rehabilitated from the time of birth to adult ages. The fibre growth retardation of the motor ventral spinal root was readily recovered by early initiated rehabilitation Hedley Whyte (1973) claimed that myelination of sciatic nerve fibres in under nourished rats is only partially restored by rehabilitation. A partial restoration of the total fibre diameters of the sciatic nerve was also found by the author (Sima, 1974) This was interpreted as presumably being due to the summation of the in principle different effect of rehabilitation on the fibres of the ventral and dorsal spinal roots both of which merge into the mixed sciatic nerve

The present study concerns the fibre population of the ventral and dorsal spinal roots of the fifth lumbar segment and of the optic nerve in normal undernourished, and nutritionally rehabilitated rats. The fibres are characterized by their axon circumference axon diameter calculated from the axon cross-sectional area, and by their number of myelin lamellae The first and the last mentioned parameters used in the current study for classifying myelinated fibres, provide a sensitive procedure for the analysis of the relationship between axonal growth and myelin deposition (Friede and Samorajski, 1967) The axon diameter estimated from the axon

cross-sectional area was also related to the number of myelin lamellae for comparison with the above mentioned relationship

The aim of the present investigation was to examine whether undernutrition affects the axonal growth and the myelin deposition differently in pure motor and in pure sensory nerve tracts furthermore to study what effect nutritional rehabilitation will have upon these two fundamental structures of a nerve fibre.

II MATERIAL AND METHODS

Animals

Virgin rats of the Spraugе Dawley strain 3 months old were mated with male rats of various ages. The time of fertilization was determined by vaginal smears twice daily All litters from which rats were examined were adjusted to 8 pups at the time of birth Only male rats were examined in the present study Control and undernourished rats were examined at the age of 90 and 180 days. Prenatally undernourished rats nutritionally rehabilitated from birth to the age of examination were sacrificed at 90 and 180 days of age Altogether 6 animals were investigated The spinal roots were examined from 90-day-old animals and the optic nerve from 180-day-old rats.

Animal environment

All control rats and rats of various experimental groups were kept in plastic cages at a temperature of 25°C and in a relative humidity of 60 per cent. The room in which the rats were kept was illuminated from 6 a.m. to 6 p.m.

Diet

All rats were given pellets with a protein content of 18,8 cal per cent concentrated fish protein (949A Astra-Ewos, Sodertälje Sweden) and water ad libitum. The content of vitamins and salt constituents was above the minimum requirement This was also true for the reduced amount of pellets given to rats subjected to undernutrition (cf Sima, 1974).

Undernutrition

The technique of undernutrition was that described by Chow and Lee (1964) This technique implies pre- and postnatal undernutrition through a maternal diet restriction amounting to 50 per cent of the amount consumed by a control mother The nutritional resticiton was initiated on the first day of gestation and lasted during pregnancy and lactation period. After weaning, the 8 pups were also subjected to a nutriment resticion of 50 per cent of the amount consumed by age-matched control rats. Undernutrition was extended to the age of investigation. All undernourished rats were allowed water ad libitum.

Nutritional rehabilitation

Prenatally undernourished litters were adjusted to 8 pups at the time of birth. From the first day post partum the mothers of these litters were allowed pellets ad libitum until weaning After weaning the 8 pups had free access to pellets until they were sacrificed at 90 and 180 days of age Rehabilitated rats were allowed water ad libitum

Histological technique

The animals were anaesthetized with ether They were perfused with a mixed solution of 1 0 per cent paraformaldehyde and 1.25 per cent glutaraldehyde in 0 15 M cacodylate buffer of pH 7 15 with added $CaCl_2$ The amount used was 1.5–2.0 ml per 1 gram body weight. This was followed by perfusion with the same volume of solution four times the above mentioned concentrations. The nerves were then left in situ in the perfused animals for 4 hours, before they were dissected out. Thereafter the nerves were fixed in concentrated solution for another 12 hours and postfixed in 1 0 per cent osmium tetroxide for 2 hours, dehydrated and embedded in Epon 812 (cf Karnovsky 1965) One micron thick complete cross sections were cut with a LKB-ultrotome from the middle level of the spinal roots and from the optic nerve 3 millimeter behind the eye-ball for light microscopic orientation Immediately adjoining sections were examined in a Philips EM 200 electron microscope

Counting techniques and presentation of the material

Electron micrographs were taken from a sector varying between 40 and 45 degrees of a cross-section of the spinal roots and of the optic nerves. Random sampling was applied to every second (spinal roots) and every third (optic nerves) grid mesh across the entire sectors Total magnification of prints used for examination varied from 26 000 to 120 000 times. The magnification was controlled with the aid of a calibration suspension containing 0.5 micron large latex spheres (Ernest F Fullam Inc Schenectady N Y U.S A) which was placed on the grid before examination. Only micrographs in which all lamellae of the myelin sheath were clearly visible were analysed. The number of myelin lamellae was counted from the innermost to the outermost dense line The axon circumference measured with a map meter was only analysed in fibres cut perpendicular to their long axis as judged by circular appearances of microtubules (figure 1 and 2). The number of axons and appurtenant number of myelin lamellae examined ranged per each nerve from 63 to 168 Lamellar counts per each nerve ranged from 168 to 437 myelin sheaths Axon circumference was plotted for each nerve against the number of myelin lamellae (figures 3 to 5) Number of myelin lamellae indicating myelin sheath thickness, was illustrated in frequency distribution diagrams in intervals of 5 or 10 lamellae and expressed as percentages of the number of total myelin sheaths counted (figures 6 to 8) From axons where the circumference was determined the



Fig 1 Myelinated axon from the ventral root of 90-day-old control rat Upper left, higher magnification of the framed part of the myelin sheath. Black spheres were used f magnification co trol (20,000X and 62,000X)

cross-sectional area was also calculated with the aid of a planimeter From the value of the axon area the diameter was determined according to the formula $d= \sqrt{\frac{4A}{\pi}}$

The complete material of estimated axon circumferences, axon areas and diameters with appurtenant lamellar counts is presented in table I to IX. The additional lamellar counts are given in table X to XII

Statistical methods

The Fisher permutation test for independent two-sample case with Edgeworth s expansion was used in order to assess the difference between control and experimental rats with respect to axon circumferences and axon diameters at given numbers of myelin lamellae The X values corresponding to myelin sheath thickness



Fig. 2 Myelinated axons from the optic nerve of an undernourished 180-day-old rat. Black sphere was used for control of magnification (86,000X)

were subgrouped in groups of almost equal magnitudes. The comparisons within the subgroups were then pooled together This statistical analysis was chosen in order to avoid the following assumptions of traditional analysis (a) normal distribution, (b) linear regression and (c) homogeneous variances. For statistical evaluation of the frequency distributions of myelin lamellae test for trend in a contingency table with p-value determination by Edgeworth s expansion and correction for continuity (Odén and Wedel 1973) was used Statistical evaluation was performed with the aid of an IBM 360 computer at Gothenburg University Computing Centre

III RESULTS

Ventral root

The relationship between axon circumference and myelin sheath thickness expressed as the number of myelin lamellae showed a nonlinear relationship in the ventral root This was true for both control undernourished and rehabilitated rats. In all three nerves an S-shaped relationship could be noted Among thin fibres with the same axon circumference the myelin sheath thickness varied within relatively wide limits. In thick fibres the axon circumferance varied more than did the myelin sheath thickness (figure 3) This nonlinear relationship was more pronounced when the axon diameter calculated from the axon cross-sectional area was related to the myelin sheath thickness In normal and rehabilitated rats no fibres were observed in the ventral root containing more than 140 lamellae In the undernourished rat the thickest myelin sheaths of the ventral root contained 130 lamellae Undernourished and rehabilitated rats showed significantly ($p<0.05$) thinner myelin sheaths in proportion to axon circumference when compared to the control rat This was also true when myelin sheath thickness was related to axon diameter No significant difference could be obtained between undernourished and rehabilitated rats in the relation of myelin sheath to axon circumference or to axon diameter (figure 3d)

In a representative part of all myelinated fibres of the ventral root the myelin sheath thickness varied between 11 and 140 lamellae in the control rat Two distinct peaks could be noted in the frequency distribution diagram (figure 6) one for myelin sheaths 41–50 lamellae thick and one for sheaths containing 121–130 lamellae

In the undernourished rat the myelinated fibres were surrounded by myelin sheaths varying from 11 to 130 lamellae thick. Even in the undernourished rat two peaks could be distinguished, one for sheaths consisting of 51–60 lamellae and one for sheaths containing 111–120 lamellae The myelin sheath thickness of the undernourished rat was significantly thinner ($p<0.001$) than in the control rat In the undernourished and subsequently rehabilitated rat no fibres were observed which contained less than 31 lamellae The thickest myelin sheaths consisted of 140 lamellae The two peaks in the frequency distribution diagram (figure 6) were contributed by myelin sheaths containing 51–60 and 91–100 lamellae Thus the rehabilitated rat did not show a full recovery of the myelin sheaths when compared to the control rat. However no significant difference could be obtained between myelin sheath thicknesses of rehabilitated and control rats. ($p>0.2$)

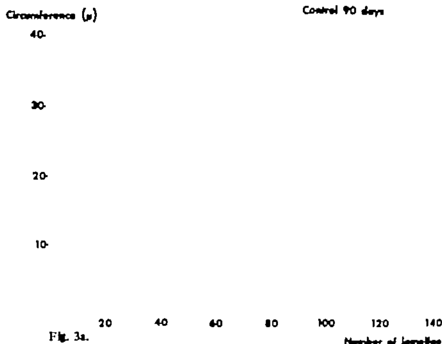


Fig. 3a.

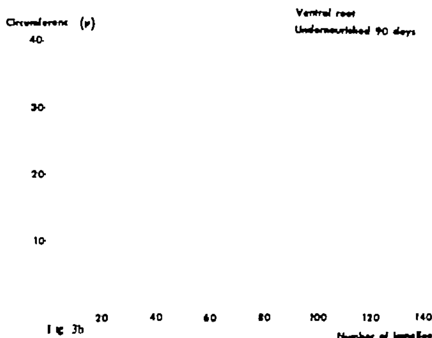


Fig. 3b

Fig. 3 Relationship between axon circumference and myelin sheath thickness, expressed as number of myelin lamellae in ventral roots. (a) Control rat (b) undernourished rat, (c) rehabilitated rat, (d) comparison between respective regression lines. These regressions were calculated without taking into account the assumptions of traditional analysis (see text). Undernourished and rehabilitated rat show significantly ($p < 0.05$) thinner myelin sheaths in proportion to axon circumferences in comparison to the control rat. No significant difference in this proportion can be obtained between undernourished and rehabilitated rats.

Ventral root
Rehabilitated 0–90 days

Circumference (μ)
40
30
20
10

20 40 60 80 100 120 140
Number of lamellae

Fig. 3c.

Circumference (μ)
40
30
20
10

Ventral root — Control 90 days
— Undernourished 90 days
— Rehabilitated 0–90 days

20 40 60 80 100 120 140
Number of lamellae

Fig. 3d.

Dorsal root

The relationship between axon circumference and number of myelin lamellae was also slightly S-shaped in the dorsal root. This nonlinear relationship was also valid for the dorsal root fibres of experimental rats (figure 4) When comparing the axon diameter with the number of myelin lamellae no strict linear relationship was noted in control or undernourished rats, nor in the rehabilitated rat. In undernourished and rehabilitated rats the proportion between axon circumference or axon diameter and myelin sheath thickness was not altered when compared with the control rat ($0.1 < p < 0.6$) See figure 4d

The myelin sheath thickness expressed as the number of myelin lamellae ranged between 10 and 130 lamellae in all three nerves. Two peaks could be distinguished in the myelin sheath frequency distribution (figure 7) both in control and in experimental rats. In the control rat these appeared for sheaths containing 41–50 and 101–110 lamellae in the undernourished rat for sheaths consisting of 31–40 and 81–90 lamellae The rehabilitated rat exhibited the peaks for sheaths consisting of 31–40 lamellae and for those made up by 91–100 lamellae Accordingly the deposition of myelin in the undernourished rat was markedly retarded In the rehabilitated rat no significant recovery could be observed as far as myelin deposition was concerned. When compared to the control rat the myelin sheath thickness of the dorsal root fibres of the rehabilitated rat was significantly thinner ($p < 0.001$)

Optic nerve

In this central nerve tract the relatinship between axon circumference and the number of myelin lamellae was almost linear in control and rehabilitated rats. In the undernourished rat the axons surrounded by the same number of myelin lamellae varied within a wide range concerning their circumferences and the linear relationship was less pronounced (figure 5) The axon diameter calculated from the axon area, was related in the same way to the myelin sheath thickness in control and rehabilitated rats as was the circumference In the undernourished rat however the linear relationship was distorted and the nerve fibres with the same number of myelin lamellae showed great variations in axon diameters. Comparing the optic nerves of control and of undernourished rats (figure 5d) the fibres of the undernourished rat showed disproportionately thicker myelin sheaths when compared with the fibres of the control rat ($p < 0.01$) Only in the largest fibres containing 30 myelin lamellae or more the myelin sheath thickness of the undernourished rat showed almost normal proportions towards the axon circumference and axon diameter In the rehabilitated rat the myelin sheath thickness was disproportionately thicker in both thin and thick fibres ($p < 0.01$) (figure 5d)

The lamellar counts of the optic nerves showed a unimodal distribution in both control and experimental rats (figure 8) In all three nerves the peak in the frequency distributions was made up by sheaths consisting of 6 to 10 lamellae In control and rehabilitated rats the myelin sheath thickness ranged between 1 and 46

lamellae. In the undernourished rat the thickest sheaths consisted of 35 lamellae. The myelin deposition was significantly impaired in the undernourished rat ($p<0.001$) as compared with the control rat, whereas in the rehabilitated rat this impairment in myelin deposition was completely recovered.

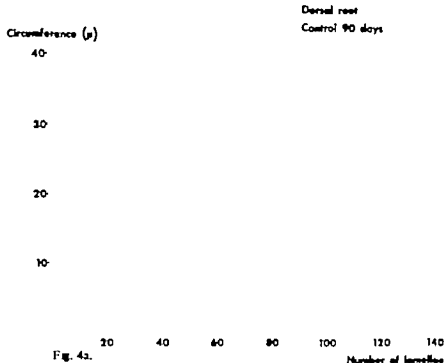


Fig. 4a.

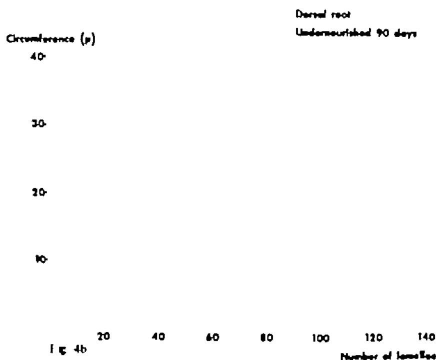
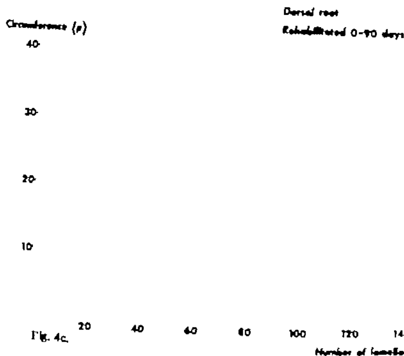


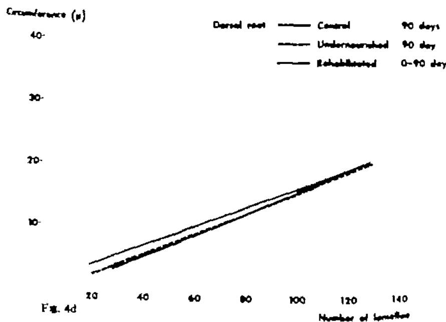
Fig. 4b

Fig 4 Relationship between xon circumference and myelin sheath thickness, expressed as number of myelin lamellae in dorsal roots ( ) Control rat (b) undernourished rat (c) rehabilitated rat, (d) comparison between respective regression lines These regressions were calculated without taking into ac ount the assumptions of traditional analysis (see text) No significant difference can be obtained between the proportion axo circumference t myelin sheath thickness in any of the nerves



Dorsal root
Rehabilitated 0-90 days
Circumference (μ)
40
30
20
10
20
40
60
80
100
120
140
Number of lamellae

Fig. 4c.



Circumference (μ)
Dorsal root
Control 90 days
Undernourished 90 day
Rehabilitated 0-90 day
40
30
20
10
20
40
60
80
100
120
140
Number of lamellae

Fig. 4d

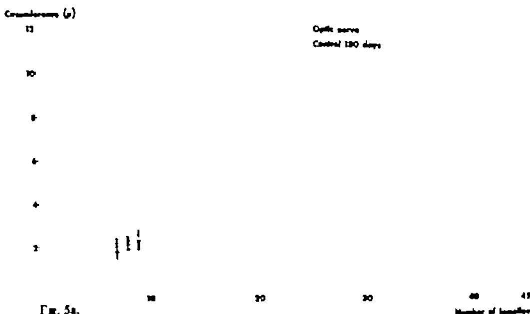


Fig. 5a.

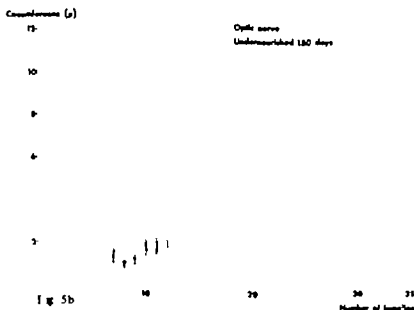


Fig. 5b

Fig. 5 Relationship between xon circumference and myelin sheath thickness in optic nerves (a) Control r t, (b) undernourished rat (c) rehabilitated rat (d) comparison between respective regression lines. These regressions were calculated without taking into account the assumptions of traditional analysis (see t xt) Undernourished and rehabilitated rats show significantly ($p<0.01$) lesser axon circumferences in proportion to myelin sheath thicknesses, in comparison to the control r t No significant difference in thi proportion can be obtained between undernourished and rehabilit ted at

Circumference (μ)

12

10

8

6

2

Optic nerve

Rehabilitated 0–180 days

10 20 30 40

Number of lamellae

Fig. 5c.

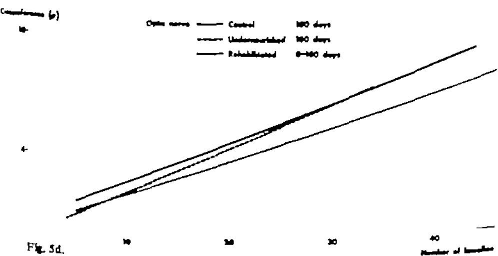


Fig. 5d.

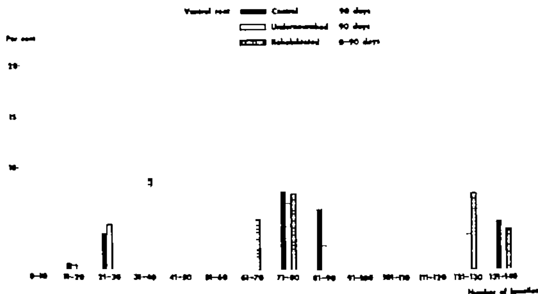


Fig. 6 Frequency distribution of pooled myelin sheath thicknesses in ventral roots. The myelin sheat thicknesses are significantly thinner ($p<0.001$) in the undernourished rat as compared to the control rat. No significant difference could be obtained between rehabilitated and control r ts. Note the bsence of thin myelin sheaths of the rehabilitated rat

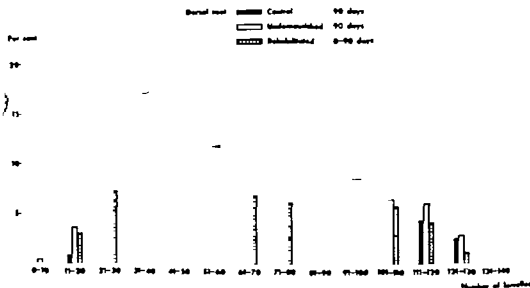


Fig. 7 Frequency distribution of pooled myelin sheath thicknesses in dorsal roots. The myelin sheath thicknesses f ndernourished and rehabil tated t re significantly thinner ($p<0.001$) than those of the control t

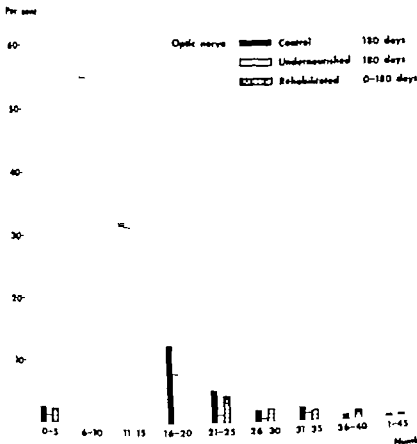


Fig. 8. Frequency distribution of pooled myelin sheath thicknesses in optic nerves. In the undernourished rat the myelin sheath thicknesses are significantly thinner ($p < 0.001$) than in the control rat. No difference can be noted between myelin thicknesses in rehabilitated and control rats.

IV DISCUSSION

Pre and postnatal undernutrition showed to have retarding effects upon the deposition of myelin in ventral and dorsal spinal roots and in the optic nerve In the ventral root the impairment in myelin deposition was almost entirely restored after nutritional rehabilitation but in the dorsal root no significant recovery could be detected after rehabilitation In the optic nerve this retardation in myelination was fully recovered by nutritional rehabilitation initiated at birth. Hedley Whyte (1973) demonstrated that severe food deprivation during the suckling period affects the myelination in the rat sciatic nerve adversely and that this deficit in myelin deposition showed to be partially reversible during subsequent nutritional rehabilitation When it is taken into account that the ventral and dorsal roots of the fifth lumbar segment merge into the sciatic nerve the results concerning the spinal roots are in agreement with those of Hedley Whyte Hence the findings in the sciatic nerve by Hedley Whyte may represent a summation of the effects of undernutrition and subsequent rehabilitation on myelin deposition of the ventral and dorsal roots.

The deficit in myelination found in the sciatic nerve must accordingly have been initiated during the suckling period. In the present study the rats were subjected to a prenatal undernutrition and rehabilitated from birth through food ad libitum to the lactating mother Therefore the irreversible deficit found in the deposition of myelin in the dorsal root may have been initiated before birth. During both late fetal life and early suckling period Schwann cell proliferation and subsequent commencement of myelination is known to occur (Asbury 1967 Friede and Samorajski 1968) Therefore nutritional deprivation initiated during fetal life or during early suckling period may affect Schwann cell proliferation and early myelination, since it is known that periods of active cell proliferation is most vulnerable towards undernutrition (Dobbing 1968)

In the histogram of myelin sheath distribution of the ventral root it is apparent that the rehabilitated rat lacks thinly myelinated fibres when compared to control and undernourished rats. This phenomenon was also observed to a lesser extent in the sciatic nerve fibres of rehabilitated rats by Hedley Whyte (1973). A plausible explanation to this phenomenon given by Hedley Whyte is that undernutrition may decrease the rate of mitosis of Schwann cells and when rehabilitation is initiated Schwann cells with myelinating axons proceed with myelination but no new axons will be myelinated. In the dorsal root thinly myelinated fibres were observed also in the rehabilitated rat This difference between ventral and dorsal spinal roots of rehabilitated rats is not known A possible explanation may be that the period of Schwann cell multiplication in the dorsal root is more prolonged than in the ventral

root, since it is known that the fibre calibre growth proceeds at a slower rate in the dorsal than in the ventral root (Sima 1974) The rate of mitotic activity of Schwann cells may therefore not be so severely affected by undernutrition as assumed for the ventral root.

A linear relationship between axonal circumference and myelin sheath thickness in normal developing rat sciatic has been demontrated by Clos and Legrand (1970) Friede and Samorajski (1968) and by Hedley Whyte (1973). However Webster (1971) and Webster and O'Connell (1970) found that the axon diameter was not proportional to the myelin sheath thickness. In the present study no linear relationship was found between axon circumference or axon diameter estimated from the axon cross-sectional area and the myelin sheath thickness in the ventral or in the dorsal root Differences may thus exist in the mode of myelin deposition in the sciatic nerve on one hand and in the ventral and the dorsal roots on the other hand. It may therefore be assumed that axonal growth is not the only factor controlling the myelin deposition at least in the ventral and dorsal roots as proposed by Friede (1972) for the sciatic nerve

In the ventral root the deposition of myelin was more affected by early instituted protein-calorie undernutrition than the axonal expansion Even in the rehabilitated rat the myelin sheaths were thinner in proportion to the a on circumference when compared to the control rat which may indicate an early reduced myelinating potential of the Schwann cells of the ventral root This is in agreement with the findings by Hedley Whyte (1973) which demonstated that the myelin sheath growth of sciatic nerve fibres was retarded to a great r extent than the axonal growth in undernourished rats. In the dorsal root, however the myelin sheath and the axon circumference were affected proportionately in both undernourished and rehabilitated rats when compared to the control rat

In the optic nerve fibres the myelin deposition was significantly retarded by undernutrition This retardation was completely recovered by nutritional rehabilitation initiated at the time of birth Such a restoration of the myelin sheath was also expected, since it is known that the oligodendrocytes initiating the myelination first appear just prior to the time when myelination begins at day 7 to 9 postnatally (Vaughn, 1969) and therefore the differentiation of oligodendrocytes would not be affected by undernutrition during the prenatal period. Between axon circumference and myelin sheath thickness there existed an almost linear relationship in the optic nerves of control and rehabilitated rats. In the undernourished rat this relationship was distorted with a wide spread in the proportion between axon circumference and myelin sheath thickness in individual fibres. In both undernourished and rehabilitated rats the axonal expansion was more affected than was the myelin sheath. Thus in the optic nerve the converse relationship was valid concerning the effect of undernutrition upon axonal growth and myelin sheath when compared to the ventral motor root in which the myelination was more affected than the axonal growth by undernutrition

When comparing the relationship between axon circumference and myelin sheath thickness on one hand and th t between axon diameter and myelin sheath

thickness on the other hand no marked differences were at hand in any of the three nerves investigated indicating that the perpendicularly cut axon may be regarded as roughly circular

The results of the present study fit well with earlier investigations on total fibre diameters in undernourished and rehabilitated rats (Sima, 1974 Sima and Sourander 1974) In these studies it was found that the calibre growth of the dorsal root and the optic nerve were irreversibly retarded by undernutrition whereas the impairment of the ventral root fibre growth was fully restored by nutritional rehabilitation. Only in the optic nerve does the complete restoration of the myelin deposition contrasts with the irreversibly retarded total calibre growth This difference may be explained by the fact that the thickness of the myelin sheath of an optic nerve fibre only constitutes a very small percentage of the total fibre diameter Therefore the difference in the myelin sheath thickness between undernourished and rehabilitated rats may not have been detected by the method used in the earlier study (Sima and Sourander 1974)

The cause of the different effect of nutritional rehabilitation on myelination in the ventral and dorsal roots of previously undernourished rats remains obscure One may speculate on differences in the mode of Schwann cell proliferation and early myelination and assume that these processes are affected differently by undernutrition in the ventral and the dorsal roots. This difference in susceptibility may perhaps be mediated by factors like different timing of Schwann cell proliferation in relation to birth or by differences in enzymatic patterns or in hormonal response of the Schwann cells. It is known that hypothyroidism may cause retardations in cell proliferation for instance in the cerebellum (cf Eayrs and Taylor 1951 Legrand, 1967) This latter mechanism has however been denied as a cause of retardation in Schwann cell proliferation and myelination (Clos and Legrand, 1970) Hypothyroidism is not conceivable as being the underlying cause in the present study since the thyroid essentially develops just after birth in the rat (cf Miller 1969). However other regularitory mechanisms, like for instance the role played by insulin which can be secreted already during the midgestation period in the fetal rat must be considered when pondering on the cause of this topographic difference in myelination of undernourished rats.

V SUMMARY

The effect of pre and postnatal undernutrition on myelin deposition and axonal radial growth was examined ultrastructurally in the motor ventral root and the sensory dorsal root of the fifth lumbar segment in adult rats. In addition the same effect was studied on the corresponding structures of adult rat optic nerve fibres. In order to evaluate whether achieved deficits were recoverable nutritional rehabilitation was initiated at birth From the results it is concluded that

1 Pre and postnatal undernutrition causes impairments in myelin deposition in ventral and dorsal root fibres of the fifth lumbar segment (LV) and in the nerve fibres of the optic nerve
2. The deficit in myelination is completely recovered in the optic nerve fibres and almost entirely restituted in the ventral root fibres of the fifth lumbar segment (LV) In the nerve fibres of the dorsal spinal root of the same segment the deposition of myelin is irreversibly distorted by early undernutrition
3 In the ventral root nerve fibres (LV) the myelin deposition is proportionately more affected by undernutrition than the axonal expansion The relation axonal radial growth to myelin deposition is affected proportionately by undernutrition concerning the nerve fibres of the dorsal spinal root (LV) In the nerve fibres of the optic nerve the axonal radial growth is proportionately more affected by pre and postnatal undernutrition than the depositon of myelin

Furthermore it is suggested that differences may exist in the mode of Schwann cell proliferation in the ventral and dorsal spinal roots (LV) of the rat.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Ass. Prof Patrick Sourander for his great personal interest and valuable criticism To Miss Kristina Müntzing, Miss Margareta Persson and Miss Hermengild Schucht my sincere thanks for their skilful technical assistance To Fil. Kand. Nils-Gunnar Pehrsson my thankfulness for efficient statistical work. This study was supported by a grant from the Swedish Medical Research Council, project No B74–12X–3488–03C

VI REFERENCES

Asbury A K Schwann cell proliferation in developing mouse sciatic nerve A radioautographic study J Cell Biol 1967 34 735–743

Bass, N.H., Netsky M G and Young, E Effect of neonatal malnutrition on developing cerebrum II Microchemical and histologic study on myelin formation in the rat Arch Neurol 1970 3 303–313

Chow B F., and Lee C J Effect of dietary restriction of pregnant rats on body weight gain of the offspring J Nutr 1964 82 10–18

Clos J and Legrand J Influence de la deficience thyroidienne et de la sous-alimentation sur la croissance et la myelinisation des fibres nerveuses de la moelle cervicale et du nerf sciatique chez le jeune rat blanc. Arch Anat Micr Morph Exp 1969 58 339–354

Clos, J and Legrand J Influence de la deficience thyroidienne et de la sous-alimentation sur la croissance et la myélinisation des fibres nerveuses du nerf sciatique chez le jeune rat blanc Étude au microscope electronique Brain Res. 1970 22 85–297

Dickerson J W T Dobbing J and McCance R A The effect of undernutrition on the postnatal development of the brain and cord in pigs. Proc Roy Soc Biol 1967 166 396–407

Dickerson J W T and Walmsley A L The effect of undernutrition and subsequent rehabilitation on th growth and composition of the central nervous system of the rat Brain 1967 90 897 906

Dobbing, J The influence of early nutrition on the development and myelination of the brain Proc Roy Soc Biol 1964 159 503 509

Dobbing J Vulnerable periods in developing brain In Applied neurochemistry

| Davison A N and Dobbing J Eds Philadelphia F A Davis Co 1968 pp 87 316

Dobbing J Undernutrition and the developing brain the use of animal models to elucidate the human problem. In Chemistry and brain development Paoletti, R., and Davison A N Eds New York Plenum Press 1971 Vol 13 pp 399–412.

Eayrs J T and Taylor S.H The effect of thyroid deficiency induced by methylthiouracil on the maturation of the central nervous system J Anat 1951 85 350 358

Friede R L Control of myelin formation by axon calibre (with a model of the control mechanism) J Comp Neur 197? 144 233–252

Friede R L and Samorajski T Relation between the number of myelin lamellae and axon cir umferen in fibres of vagus and sciatic nerves of mice J Comp Neur 1967 130 3 32

Friede R L and Samo ajski T Myelin formation in the sciatic nerve of the rat A quantitative electron microscopic histochemical and radiographic study J Neuropath Exp Ne rol 1968 27 546 570

Hedley Whyte E.T., Myelination of rat sciatic nerve comparison of undernutrition and cholesterol biosynthesis inhibition J Neuropath Exp Neurol 1973 32 284 302
Hedley-Whyte E T and Meuser C.S The effect of undernutrition on my lin tion of rat sciatic nerve Lab Invest 1971 24 156 161
Karnovsky M.J A formaldehyde-glutaraldehyde fixative of high osmol ... for use in lectron microscopy J C ll Biol 1965 27 137A
Legrand J., Analyse de l'action morphog netique d s hormones thyroidienne sur le cervelet du jeune rat Arch Anat Micr Morph Exp 1967 56 05 44
Miller S.A Protein metabolism during growth and dev lopment In M mmali n protein metabolism Vol III Munro H N Ed Academi Pr ss N w York 1969
Oden A and Wedel H Arguments for Fisher s permutation t st Dept of M th Chalmers Institute of Technology Göteborg No 1973 14 1973
Sima, A., Studies on fibre size in developing sciatic nerve and spinal roots in normal undernourished and rehabilitated rats Acta Physiol Scand Suppl 406 1974
Sima A Olsson Y and Sourander P Experimental observations in periphe al nerves in undernourished rats. Acta Path Microbiol Scand (A) 1971 79 310
Sima A and Sourander P The effect of early undernutrition on the calibre spectrum of the rat optic nerve 1974 To be published in Acta N u opath (Berl)
Vaughn J E An electron microscopic analysis of gliogensis in rat optic nerv Z Zellforsch. 1969 94 293–324
Webster H de F The geometry of peripheral myelin sheaths du ng their formation and growth in rat sciatic nerves J Cell Biol 1971 48 348 367
Webster H de F and O'Connell M F Myelin formation in peripheral nerves A morphological reappraisal and its neuropathological significance In VIth International Congress of Neuropathology Paris 1970 Masson t Cie pp 579–588

VII TABLES

Table I Ventral root of control rat 90 days old

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
17	3.61	1.00	1 13	80	8 00	4 11	2.29
29	5 43	2 09	1.63	83	8 65	4.59	2 42
29	3.64	0 95	1 10	88	26 00	31.26	6.31
30	4 15	1.31	1.29	90	16 00	15.68	4 47
31	5.36	2.06	1.62	91	17 40	14 41	4.28
32	8.40	3 93	2.24	91	12..0	7.26	3.04
33	7 14	3.35	2 07	93	13.20	10.28	3.62
35	4 91	2.08	1.63	97	8 40	3.25	2 03
35	6 00	1 92	1.56	97	16 90	15 41	4.39
36	3.29	0.73	0.96	98	9.60	6.35	2.84
38	8 38	3 94	2.24	98	10 40	5.65	2.68
38	16 40	18 12	4.80	99	9.60	5.02	2.53
40	3 17	0 74	0 97	102	22.00	29.35	6 11
41	6 43	2.57	1.81	104	8 40	3.06	1 97
41	9 71	4.97	2.52	104	10 40	4 11	2.29
43	7.24	2 96	1 94	104	19.80	22.53	5.36
45	7.00	3 16	2 01	108	23.20	36 76	6.84
48	9 60	5 90	2 74	108	15.20	14.20	4.25
50	5 79	1 76	1.51	110	22 40	34.25	6.60
51	5.00	2.28	1 70	110	13.93	13.53	4 15
57	11 60	5.31	2.60	110	18.80	2.93	5 40
58	7.57	3 43	2 10	111	18 00	18 79	4.89
58	9 80	5 68	2.69	115	25.00	42 16	7.33
59	13.20	5 73	2 70	116	22.80	30.34	6.22
63	6 43	3 01	1 96	121	16.80	18.93	4 91
69	11 11	5.07	2.54	122	17 78	17 16	4.68
74	12 00	7.01	2.99	125	16 61	20 45	5 10
76	6 18	2 49	1 78	125	27.20	4 .83	7.39
76	15 20	8.28	3.25	130	20.40	27.00	5.86
76	8 40	3 48	2 11	131	2..00	32 40	6 42
77	11.60	6.29	2.83	131	16 41	17.07	4.66
79	11 20	5 92	2 74	134	13.39	13 48	4 14

Table II Ventral root of undernourished rat 90 days old

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
18	3.38	0 82	1 01	38	4.60	2.70	1.85
2	6 63	2 37	1 75	44	7 00	2.33	1 71
23	4.00	1 00	1 13	44	7 67	4.62	2 42
25	14 00	5 03	2.52	45	6.80	2 00	1.60
8	5 60	1 40	1 34	51	5.00	2 04	1.60
30	4 80	1.60	1 43	53	5 60	1 90	1 56
34	6 00	2 93	1 92	53	21 20	1.81	5.27
35	9 40	2 57	1 82	54	6 80	2 90	1 92
35	4 67	1 72	1 47	60	6.20	1.60	1 43
35	6 67	1 76	1 51	60	7 60	4.00	2..6
36	1 60	3 95	2 26	62	6 67	3 13	1 99
36	5 00	1 89	1 56	65	18 40	8..0	3 3
36	4 75	1 26	1 29	65	8 00	3 90	2..3
37	7.33	13	1 64	65	6 40	2 43	1 75

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Are μ^2	Diameter μ
70	10 80	6.80	94	114	13.67	15.82	4 49
77	10.00	5 40	2.6	114	28 40	48.89	7.89
82	7.60	3.30	2.05	115	21.33	37 96	6.96
88	19 17	17.61	4 74	115	29.60	58 90	8.66
91	15.20	13.30	4 12	117	25.60	36 63	6 83
95	20.40	26.80	5.84	118	20.80	30 20	6.20
99	20.00	24.59	5.60	118	18.17	26_4	5 78
100	16.80	18.80	4.89	118	22.00	32.00	6.38
101	17.00	21 17	5.20	118	19.50	8.02	5 97
102	20.00	26 43	5.80	118	21 00	35 78	6 75
107	19.00	30.87	6.27	119	26 80	39 47	7 09
110	19 00	27.08	5.88	119	20.83	9 73	6 15
110	18 67	28.89	6 07	121	22 00	34.80	6 66
110	18 67	28 19	5 99	121	23 20	36 70	6.84
112	22.80	30.80	6_6	123	29.20	56 70	8 50
112	28.40	50.55	8 03	126	25 40	41.20	7.24
113	23.60	35.60	6 73				

Table III. Ventral root of rehabilitated rat, 90 days old.

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Are μ^2	Diameter μ
31	3.20	0.60	0.87	62	18.00	24 60	5.60
32	7.60	3.90	2.23	63	13 40	8 10	3.21
33	3 70	1 10	1 18	64	7.20	3.30	2.05
34	7.60	3 10	1 99	65	10.40	6 70	2.92
36	7 40	2.80	1 89	66	9 70	7.00	2.99
36	10 00	4 00	2.26	69	13.20	6 10	2 79
38	6.30	1.50	1.38	71	8.30	5 50	2.65
40	12.00	3.00	1.95	72	14.00	8 90	3.37
40	8 00	4.38	2.36	72	11.60	7.90	3 17
40	9 40	5.90	2 74	78	13 60	6.20	2.81
41	6.50	2.60	1 82	79	24.80	33.80	6.56
41	6.33	3 30	2.05	80	10.30	6 10	79
43	7 00	3 40	2 08	85	9 60	7 10	3 01
45	10.20	5.00	2 52	87	9.50	8.70	3 33
46	8.00	4.60	2 42	87	15 20	8.30	3 5
46	7.20	3.60	2 14	90	17.80	19 00	4.92
48	11.20	6 70	2 92	91	13.80	13.20	4 10
48	7.60	4 10	2.29	92	21.60	27.60	5 93
48	8.80	4 10	2 29	93	11 67	11.60	3 84
52	12.70	6.60	2 90	94	17.30	23 80	5 50
52	11.20	5 60	2 67	95	20 40	27.80	5 95
55	6.80	3 00	1.95	100	25.80	38 10	6.97
57	9.20	5.00	2.52	101	22.60	33.20	6 50
58	7.00	4 10	2 29	101	26.80	44 70	7 55
58	9 00	5 80	2 72	102	22 00	33.90	6 97
59	9 20	4 40	2.37	103	17.00	24 70	5 61
60	12.00	8 50	3.29	103	20.80	30 70	6.25
60	11 70	7 60	3 11	103	23.80	36.80	

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
103	25.80	42 50	7.36	114	26 40	46.80	7 72
104	25.20	39.60	7 10	114	22.00	33 00	6 48
107	21.00	27.50	5 92	116	22.80	32 60	6 44
111	25.20	43.80	7 47	116	21.00	36 70	6.84
111	12.30	11.60	3 84	118	22.80	36.10	6 78
111	21 20	29 00	6 08	119	20 40	28.80	6 06
112	2_.80	33 40	6.52	119	27.60	52.50	8 18
11	15.20	14.80	4.34	125	22 80	33 10	6.49
114	31.20	63.20	8 97				

Table IV Dorsal root of control rat, 90 days old

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
28	4 00	1.36	1.34	88	12.60	7.30	3 05
34	12.00	11 65	3.86	89	15.60	16.60	4.60
34	4 00	1.50	1.38	94	8 80	5.59	2.67
35	4 00	1 44	1.34	95	10 67	7 40	3.07
38	7.60	3.27	.05	97	10.67	9 01	3.39
39	5.60	1.92	1.56	97	12 20	8.30	3.25
39	4 10	1 33	1 29	99	9.00	5.30	2.60
40	7._0	3 40	2 08	99	16.20	15 10	4.39
41	5._0	1 56	1 43	99	18 00	22 90	5 40
42	4.67	1.89	1.56	99	12.80	10.50	3.66
43	6.60	2 00	1.60	100	11 00	10.04	3.57
43	5 80	2.30	1 71	100	20 00	31.51	6.33
44	5 60	1 94	1.56	101	12 20	9 91	3.55
45	6 00	1 97	1.60	101	12 83	12 20	3 94
45	6.60	2 10	1.64	102	14 60	15 30	4 41
47	6 00	2 68	1 85	103	21.33	26 79	5.84
48	8 40	2 59	1 82	103	29 80	20 50	5 11
49	5 33	1 34	1 29	103	22 00	29 78	6 16
[illegible]9	6 00	1 47	1 38	104	26 57	42 04	7.31
[illegible]	9 00	3 60	2 14	106	13 67	14.86	4 36
5[illegible]	4 40	1 29	1 29	106	16 40	14.80	4.34
5	6 40	2 34	1 71	106	14.60	12.80	4 04
53	6 00	2 50	1 78	106	15 00	15.60	4 46
53	11 33	5 34	2 60	107	16 00	19 77	5 02
55	6 80	75	1 89	109	17 00	17 74	4 75
59	8 80	4 70	2 45	110	24 40	36.21	6 79
61	10 80	6 10	2 79	111	2.60	27.00	5.86
67	7 80	3 77	2 20	112	17 40	18 91	4 91
70	6 67	3 69	17	114	19 00	29.21	6.10
80	10 83	10 22	3 60	120	15 80	16 40	4.57
82	8 80	5 19	57	121	24.00	39.67	7 11
83	10 57	6 2	83	1 3	22 33	44 52	7.53
86	1 40	8 90	3 37	1 3	24 00	34 90	6 67
86	11 60	9 50	3 48	124	3.80	38 91	7.04
85	11 33	10 5	3 66	127	17 20	0.58	5 1
88	9 80	6 50	2 88				

Table V Dorsal root of undernourished rat, 90 days old

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
18	3.00	0.55	0.87	92	13.40	11.17	3.78
19	3.50	1.03	1.13	92	13.40	11.09	3.76
20	2.60	0.55	0.87	93	9.80	5.77	2.72
25	3.60	0.96	1.13	94	11.80	8.58	3.31
32	4.83	1.73	1.47	94	16.60	18.91	4.91
32	4.00	0.55	0.87	94	17.75	14.84	4.34
33	4.60	1.11	1.18	97	11.40	8.11	3.21
37	5.00	1.95	1.60	98	13.20	11.76	3.88
38	4.80	1.70	1.47	98	12.80	11.32	3.79
38	6.00	2.43	1.75	98	15.40	16.57	4.60
42	10.00	2.22	1.67	98	15.40	16.57	4.60
47	6.20	1.75	1.51	99	11.60	9.44	3.46
51	13.80	10.30	3.62	103	17.20	18.38	4.84
57	8.60	4.94	2.50	103	16.60	17.97	4.79
67	11.20	6.67	2.92	103	17.40	22.40	5.34
68	11.40	6.86	2.96	104	21.20	25.44	5.69
69	9.00	4.66	2.45	107	9.60	5.50	2.65
69	9.40	4.77	2.47	107	12.80	9.54	3.48
73	10.80	6.64	2.90	109	21.20	29.23	6.10
73	11.80	8.64	3.31	111	21.80	20.53	5.11
76	9.60	6.48	2.88	112	24.60	33.83	6.56
76	11.00	7.81	3.15	112	14.80	14.62	4.31
78	9.00	3.96	2.26	113	18.80	24.82	5.62
79	9.80	6.14	2.79	115	15.50	21.29	5.21
80	10.67	8.75	3.34	115	23.20	29.05	6.09
82	10.20	6.01	2.76	117	16.17	22.90	5.40
83	11.00	7.87	3.17	117	18.80	21.09	5.18
84	11.00	7.83	3.16	117	22.60	35.50	6.72
86	12.40	10.21	3.60	118	20.60	20.38	5.10
86	13.40	12.54	3.99	119	22.00	29.12	6.09
86	14.60	11.91	3.89	121	17.00	19.94	5.03
88	15.40	15.47	4.44	123	21.40	30.18	6.20
88	11.00	8.42	3.27	124	27.80	50.28	8.00
88	8.40	5.33	2.60	124	17.60	16.46	4.58
90	16.40	17.65	4.75	124	20.60	26.30	5.79
90	11.40	8.51	3.29	128	22.20	32.54	6.43

Table VI Dorsal root of rehabilitated rat, 90 days old

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
19	3.70	1.40	1.34	41	8.40	2.00	1.60
30	3.30	0.90	1.07	41	7.20	3.10	1.99
32	4.40	2.60	1.82	41	5.00	1.40	1.34
38	9.60	2.70	1.85	41	8.40	3.70	2.17
40	4.00	1.30	1.29	44	11.00	3.00	1.95
40	6.30	1.60	1.43	49	11.60	7.90	3.17
40	5.60	1.70	1.47	50	9.50	6.10	2.79
40	5.20	1.80	1.51	54	11.20	6.00	2.76

Number of lamellae	Circum-ference μ	Area μ	Dia-meter μ	Number of lamellae	Circum ference μ	Area μ^2	Dia-meter μ
57	9.20	3 10	1 99	100	17.20	19.60	5.00
67	1 40	6 90	2.96	100	16.00	15.50	4 44
71	11 20	5.80	2 72	101	12.40	9 40	3 46
7	10 00	7.30	3 05	101	20.40	20.60	5 12
73	11_0	9.30	3 44	103	17.20	18 70	4.88
76	11.20	7.50	3 09	103	11.30	10.90	3 73
77	12.80	8 10	3.21	104	16.30	20.20	5.07
8	14 00	14 40	4.28	105	14 00	15 10	4.39
82	12 00	8 10	3_1	107	16 00	15.10	4.39
82	16.80	18.30	4.83	107	19.20	21 10	5 18
83	11.20	7.20	3 03	108	18.40	22 40	5.34
84	14 40	12 10	3 93	108	16.80	19.20	4.95
87	27 0	14.60	4.31	109	19.60	25.50	5 70
90	14 40	13 70	4 18	110	21 40	28.80	6.06
91	11.60	8.30	3.25	111	22 40	30.00	6 18
9	1_40	9 70	3.5	111	13 70	14.60	4.31
92	14 40	13.50	4 15	112	22.00	31 70	6.35
93	13.60	10 70	3.69	112	18.80	22.60	5.37
93	19 40	21.50	5.23	112	16.80	24 00	5.53
93	20 00	20.50	5 11	112	21 00	35 20	6.68
96	12.20	12.10	3 93	114	16 40	17.00	4.65
96	13 00	13.50	4 15	116	22.00	27.60	5 93
97	15 80	16 70	4.61	121	19.60	23.60	5 48
98	14 40	11.80	3.88	123	18.30	28.20	6.00
99	11 70	9.50	3 48	123	22.60	31 70	6.35
99	13.20	13 30	4 12				

Table VII Optic nerve of control rat, 180 days old

Number of lamellae	Circum ference μ	Area μ^2	Dia-meter μ	Number of lamellae	Circum-ference μ	Area μ^2	Dia-meter μ
5	1 73	0_1	0.52	7	2.36	0 43	0 74
5	2 00	0 32	0.64	7	2.82	0.63	0 90
5	1 81	0 20	0.50	7	2.05	0.30	0 62
5	1 62	0 1	0.52	7	1.68	0.21	0.52
5	1 91	0 24	0.55	7	2 18	0.28	0.60
5	2 05	0 24	0 55	7	3.00	0.67	0.92
6	1 27	0 08	0.32	7	2.00	0.21	0.52
6	3 45	0 71	0 95	7	1 71	0.15	0.44
6	1 77	0.24	0.55	7	1.81	0.25	0.56
6	2 18	0.35	0 67	7	1.81	0.25	0.56
6	1.55	0 19	0 49	7	2.38	0 48	0 78
6	2 38	0 34	0.66	7	1 76	0.22	0.53
6	2 24	0 29	0 61	7	2.29	0.35	0.67
6	2 14	0 33	0 65	7	1.62	0.25	0.56
6	1 81	0 18	0 48	7	1.86	0.21	0.52
6	1 91	0 25	0 56	7	2 43	0.38	0 70
7	1 46	0 14	0 42	7	2.00	0.28	0.60
7	3	0 25	0 56	7	1 76	0 19	0 49
7	2 00	0 28	0 60	8	2 73	0 46	0 77
7	1.81	0 24	0 55	8	2.46	0.34	0 66

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
8	1.23	0 11	0.37	10	1 43	0 14	0 42
8	3.09	0 71	0 95	10	2.48	0.39	0 70
8	2.36	0 41	0 72	10	2.86	0.53	0.82
8	2 18	0.28	0.61	10	2.67	0.39	0.66
8	2.54	0.47	0 77	10	2 19	0.36	0.68
8	1 91	0.30	0.62	10	2 10	0.24	0.55
8	2.00	0.27	0.59	10	2.24	0.33	0.65
8	1.64	0.21	0.52	11	1.82	0.24	0.55
8	1.91	0.29	0.61	11	2 73	0.60	0 87
8	2.32	0.34	0 66	11	2.00	0.24	0.55
8	2.32	0.29	0.61	11	2.72	0 43	0 74
8	2.36	0.30	0.62	11	3.00	0.62	0.89
8	2.05	0.24	0.55	11	3 48	0.65	0 91
8	2.38	0.36	0.68	11	1.91	0.29	0.61
8	2.67	0 44	0 75	12	2.09	0.31	0.63
8	2.71	0 49	0 79	12	2.27	0.31	0.63
8	2 10	0.23	0.54	12	2.50	0 46	0 77
8	2.29	0 17	0 47	12	3 18	0.59	0 87
8	3 48	0.55	0.84	12	3 72	0 78	1.00
8	2.24	0.36	0.68	12	3 14	0.57	0.85
8	2.29	0.33	0.65	12	7.38	0 86	1 05
8	2.33	0.33	0.65	12	2 76	0.50	0.80
8	1.81	0.23	0.54	13	3 73	0 95	1 10
8	2 05	0.35	0.67	13	2.10	0.26	0.58
9	2.00	0.35	0.67	13	2.57	0.51	0.81
9	2.18	0.33	0.65	14	2.68	0.54	0.83
9	3 18	0.51	0 76	14	3.23	0 73	0 96
9	2.27	0.33	0.65	15	3 45	0.80	1.01
9	2 18	0.32	0.64	15	3.27	0 75	0 98
9	2 18	0.38	0 70	15	2 73	0.50	0.80
9	2.64	0 49	0 79	15	6 95	1 99	1.59
9	2.27	0.36	0.68	15	2.19	0.34	0.66
9	2.00	0.27	0.59	15	2.67	0.38	0 70
9	1.64	0.20	0.50	15	2.95	0.60	0 87
9	2.05	0.29	0.61	16	3 91	0 95	1 10
9	2.81	0.52	0.81	16	2.00	0.29	0 61
9	2.64	0 48	0 78	17	4 48	0 75	0 98
9	2 71	0.29	0.61	17	4.38	0.88	1 06
9	1 71	0 21	0.52	19	4 00	0.87	1.05
9	2.10	0.33	0.65	19	3.33	0.63	0 90
9	2.29	0.35	0.67	21	3.95	1.00	1 13
9	2.48	0 49	0 79	22	5.27	1 78	1 51
9	3.05	0.56	0.84	22	8.27	3.89	2 23
9	1 91	0 29	0 61	22	5.81	1.59	1 42
9	1 91	0.24	0.55	24	7.86	2.28	1 70
10	1.91	0.25	0.56	24	6 43	2 03	1.61
10	1.55	0.17	0 47	25	8.29	3 43	2 09
10	2.64	0 44	0.75	25	5.57	1.89	1.55
10	1 91	0.28	0.60	29	8.96	4 06	1.37
10	3 18	0.43	0 74	31	4.64	1.36	1 32
10	1 81	0.28	0.60	38	6.62	2 84	[illegible]
10	2 27	0 34	0 66	44	7 71	3 89	[illegible]
10	2 48	0 40	0 71				

Table VIII Optic nerve of undernourished rat, 180 days old.

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
4	0.86	0.04	0.23	8	1.09	0.09	0.34
4	2.14	0.33	0.65	8	1.68	0.2	0.53
4	1.05	0.06	0.28	8	1.09	0.09	0.34
5	1.45	0.15	0.44	8	1.14	0.09	0.34
5	1.82	0.17	0.47	8	1.27	0.11	0.37
5	4.09	0.72	0.96	8	1.09	0.10	0.36
6	0.50	0.02	0.16	8	1.32	0.12	0.39
6	1.41	0.17	0.47	8	1.41	0.13	0.40
6	0.82	0.06	0.28	8	0.91	0.06	0.28
6	1.73	0.23	0.54	8	1.41	0.13	0.41
6	0.91	0.05	0.25	8	1.55	0.17	0.26
6	1.41	0.13	0.41	8	2.45	0.40	0.71
6	0.95	0.05	0.25	8	1.05	0.08	0.32
6	1.77	0.18	0.48	8	0.77	0.04	0.23
6	2.64	0.21	0.52	8	1.73	0.20	0.50
6	1.18	0.09	0.34	8	2.59	0.47	0.77
6	1.64	0.21	0.5	8	1.55	0.15	0.44
6	3.64	0.57	0.85	9	1.27	0.13	0.41
6	1.86	0.20	0.50	9	1.50	0.16	0.45
6	1.27	0.11	0.37	9	1.73	0.20	0.50
6	1.09	0.08	0.32	9	1.95	0.26	0.58
7	1.32	0.14	0.42	9	41	0.23	0.54
7	1.45	0.18	0.48	9	1.27	0.14	0.42
7	2.23	0.32	0.64	9	1.36	0.13	0.41
7	3.00	0.48	0.78	9	1.09	0.09	0.34
7	0.59	0.02	0.16	9	1.91	0.22	0.53
7	0.91	0.07	0.30	9	1.23	0.11	0.37
7	2.86	0.30	0.62	9	1.18	0.11	0.37
7	1.55	0.12	0.39	9	5.55	1.26	1.27
7	3.64	0.63	0.90	9	1.73	0.11	0.37
7	1.59	0.17	0.47	9	0.95	0.07	0.30
7	1.36	0.1	0.39	9	3.64	0.77	0.99
7	1.05	0.08	0.32	9	4.05	0.78	1.00
7	1.73	0.23	0.54	9	3.00	0.48	0.78
7	1.05	0.08	0.3	9	0.73	0.03	0.20
7	1.59	0.15	0.44	9	2.09	0.27	0.59
7	1 7	0.12	0.39	9	2.73	0.47	0.77
7	3.77	0.43	0.74	9	0.95	0.07	0.30
7	1.05	0.08	0.32	10	1.91	0.25	0.56
7	1.36	0.13	0.41	10	3.09	0.35	0.67
7	2.23	0.33	0.65	10	1.36	0.14	0.4
7	1.27	0.12	0.39	10	1.68	0.17	0.47
8	2.64	0.58	0.86	10	2.09	0.25	0.56
8	2.55	0.41	0.72	10	1.50	0.13	0.41
8	2.50	0.23	0.54	10	1.23	0.13	0.41
8	0.55	0.0	0.16	10	1.68	0.01	0.11
8	0.91	0.09	0.34	10	1.95	0.27	0.59
8	1.09	0.10	0.36	10	1.55	0.19	0.49
8	2.95	0.53	0.82	10	1.77	0.19	0.49
8	1.36	0.13	0.41	10	1.00	0.07	0.30
8	0.95	0.08	0.32	10	1.77	0.25	0.56
8	1.64	0.17	0.47	10	1.95	0.26	0.58

Number of lamellae	Circumference µ	Area µ²	Diameter µ	Number of lamellae	Circumference µ	Area µ²	Diameter µ
10	1.05	0.10	0.36	13	1.77	0.24	0.55
10	1.91	0.24	0.55	13	1.36	0.14	0.42
10	1.73	0.22	0.53	13	1.95	0.29	0.61
11	1.68	0.14	0.42	14	3.18	0.75	0.98
11	1.59	0.20	0.50	14	2.59	0.45	0.76
11	1.41	0.14	0.42	14	1.77	0.25	0.56
11	1.45	0.16	0.45	14	3.23	0.69	0.94
11	4.36	0.28	0.60	14	2.77	0.54	0.83
11	2.00	2.04	1.61	14	3.55	0.67	0.92
11	1.86	0.23	0.54	15	2.77	0.58	0.86
11	3.45	0.68	0.93	15	2.27	0.31	0.63
11	1.23	0.11	0.37	15	2.95	0.46	0.77
11	1.82	0.24	0.55	15	1.18	0.11	0.37
11	1.59	0.17	0.47	16	2.14	0.31	0.63
11	1.73	0.22	0.53	16	3.45	0.62	0.89
11	2.23	0.36	0.68	16	4.05	0.92	1.08
11	1.64	0.21	0.52	16	6.00	0.73	0.96
11	2.14	0.24	0.55	16	2.00	0.30	0.62
11	3.59	0.87	1.05	16	5.45	1.48	1.37
11	1.95	0.26	0.58	17	2.59	0.47	0.77
11	1.73	0.23	0.54	17	2.45	0.26	0.58
12	1.90	0.24	0.55	18	8.55	2.59	1.82
12	3.82	0.94	1.09	21	4.50	1.25	1.26
12	1.64	0.21	0.52	22	3.91	1.00	1.13
12	1.00	0.08	0.32	23	6.09	1.57	1.41
12	1.77	0.22	0.53	24	4.91	1.56	1.41
12	0.91	0.06	0.28	29	6.41	2.23	1.69
12	2.68	0.50	0.80	30	7.86	2.37	1.74
12	2.14	0.33	0.65	32	6.68	2.16	1.66
12	1.18	0.10	0.36	32	7.18	2.36	1.73
12	1.55	0.18	0.48	34	7.14	2.74	1.87
12	1.95	0.28	0.60	34	11.55	4.14	2.30

Table IX. Optic nerve of rehabilitated rat, 180 days old.

Number of lamellae	Circumference µ	Area µ²	Diameter µ	Number of lamellae	Circumference µ	Area µ²	Diameter µ
5	1.30	0.13	0.41	7	2.13	0.33	0.65
5	1.83	0.26	0.58	7	1.04	0.07	0.30
5	1.22	0.11	0.36	7	0.61	0.03	0.20
5	2.00	0.33	0.65	7	1.09	0.10	0.36
5	1.57	0.20	0.50	7	2.17	0.40	0.71
5	1.48	0.17	0.47	7	2.17	0.36	0.69
5	1.48	0.16	0.45	7	1.57	0.19	0.49
6	2.26	0.32	0.64	8	0.87	0.06	0.28
6	1.65	0.20	0.50	8	1.91	0.26	0.58
6	1.70	0.26	0.58	8	3.04	0.49	0.79
7	1.83	0.29	0.61	8	1.83	0.27	0.59
7	1.83	0.26	0.58	8	2.00	0.28	0.60
7	1.48	0.17	0.47	8	1.39	0.14	0.4[illegible]

Number of lamellae	Circumference μ	Area μ^2	Diameter μ
8	1.30	0 14	0 42
8	1.83	0.27	0.59
8	1.65	0.20	0.50
8	2.00	0.33	0.65
8	1.65	0 19	0 49
8	1 91	0.26	0.58
8	2 09	0.33	0 65
8	1.65	0.21	0.5
8	1.83	0.23	0.54
8	0.87	0.06	0.28
8	1.30	0 13	0 41
8	2.22	0.38	0 70
8	1 74	0.23	0.54
8	1.39	0 16	0 45
8	1.57	0.21	0.52
8	1 74	0.23	0.54
8	1.35	0 15	0 44
8	1.57	0.20	0.50
9	1.91	0.27	0.59
9	1.35	0 13	0 41
9	1.83	0.20	0.50
9	1.87	0.24	0.55
9	1 91	0.29	0.61
9	1.52	0 18	0 48
9	2.00	0.31	0.63
9	1.65	0.23	0.54
9	1.57	0 18	0 48
9	1.65	0.23	0.54
9	1.87	0 12	0.39
9	2.26	0.35	0.67
9	1.57	0.20	0.50
9	1.61	0.21	0.52
9	1.22	0 14	0 42
9	1 96	0.29	0.61
9	1 74	0.22	0.53
9	1 57	0.22	0.53
9	1.65	0 15	0 44
9	1 87	0 6	0.58
9	1.52	0.[illegible]0	0 50
9	1 2	0 11	0.37
10	2 09	0 29	0 61
10	3 09	0 53	0.82
10	1 78	0 25	0 56
10	1 91	0 24	0 55
10	1 74	0 6	0.58
10	1 83	0 4	0.55
10	1 74	0 22	0.53
10	1 22	0 22	0.53
10	1 43	0 14	0 42
10	1 83	0 0	0 50
10	2 48	0 45	0 76
11	1 74	0 23	0 54
11	2 48	0 43	0 74
11	1.65	0.23	0.54
11	2.26	0.39	0 70
11	2 43	0 41	0 72
11	1 48	0 17	0.47
11	1.87	0.27	0.59
11	2.35	0 44	0.75
12	1.83	0.26	0.58
12	1.83	0.20	0.50
12	2.57	0 13	0 41
12	2 74	0.50	0.80
12	2.52	0 45	0 76
12	3 04	0.62	0.89
12	1.83	0.34	0.66
12	1.57	0 19	0 49
12	1 48	0.[illegible]0	0.50
12	2.17	0.38	0 70
12	1 74	0.23	0 54
12	2.61	0 4	0 73
13	2.26	0.33	0.65
13	2 70	0 43	0 74
13	2.61	0.53	0 82
13	1 74	0.22	0.53
13	1 96	0.31	0.63
13	1 65	0 19	0 49
13	2.61	0 40	0 71
13	2.61	0.55	0.84
14	2.17	0.38	0 70
14	2 44	0 44	0 75
14	2.61	0.51	0.80
14	2.52	0 47	0 77
14	2.52	0.50	0 80
14	1.65	0 18	0 48
14	2.09	0.33	0.65
15	3.[illegible]6	0 66	0 92
15	2 35	0.32	0.64
16	1.83	0.26	0.58
17	3.30	0.66	0 92
17	2 48	0.84	1.03
18	2 91	0.68	0 93
18	3 70	1 10	1 18
18	3 91	1.07	1 17
18	2.52	0.39	0 70
19	2.57	0.53	0 82
20	2 96	0.66	0 92
20	2.57	0.99	1 12
21	3 39	0.86	1 05
21	4.09	1.00	1 13
22	2 70	0.53	0.82
24	3.04	0 74	0 97
24	4.00	1 17	1.[illegible]2
25	4 96	1 17	1.22
28	5.83	.66	1.84
31	5.22	1 76	1.50

Number of lamellae	Circumference μ	Area μ^2	Diameter μ	Number of lamellae	Circumference μ	Area μ^2	Diameter μ
31	5.39	1.85	1.54	36	8.09	4.07	2.28
35	4.87	1.77	1.50	40	9.13	5.73	2.70
35	11.48	3.03	1.96	44	5.65	2.16	1.66
35	6.09	2.39	1.74	46	6.26	2.81	1.89

Table X. Lamellar counts of the ventral root

Rat and Age in days	Groups of pooled lamellae														Number of lamellae measured
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	
Control 90 days		1	6	14	15	11	8	13	10	18	18	15	31	8	168
Undernourished 90 days		1	9	26	22	34	20	13	5	12	17	32	7		198
Rehabilitated 90 days				18	18	21	10	15	16	31	25	22	15	8	199

Table XI Lamellar counts of the dorsal root

Rat and Age in days	Groups of pooled lamellae														Number of lamellae measured
	0–10	11–20	21–30	31–40	41–50	51–60	61–70	71–80	81–90	91–100	101–110	111–120	121–130	131–140	
Control 90 days		2	7	27	27	20	11	20	19	30	38	10	6	1	228
Undernourished 90 days		1	9	21	42	26	29	21	23	26	21	16	15	7	245
Rehabilitated 90 days		8	19	51	36	31	18	16	21	31	15	11	3		260

Table XII Lamellar counts of the optic nerve

Rat and Age in days	0–5	6–10	11–15	16–20	21–25	26–30	31–35	36–40	41–45	46–50	Number of lamellae measured
	Groups of pooled lamellae										
Control 180 days	10	162	127	50	21	8	9	3	2		392
Under nourished 180 days	6	241	139	36	6	3	6				437
Rehabilitated 180 days	10	162	134	52	18	9	7	6	2	1	401

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 411

INFLUENCE OF WORK DURATION ON THE REGULATION OF MUSCLE BLOOD FLOW

By

BRITA EKLUND

STOCKHOLM 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 411

FROM THE DEPARTMENT OF CLINICAL PHYSIOLOGY
KAROLINSKA SJUKHUSET
STOCKHOLM, SWEDEN

INFLUENCE OF WORK DURATION ON THE REGULATION OF MUSCLE BLOOD FLOW

By

BRITA EKLUND

STOCKHOLM 1974

CONTENTS

page

Part A

Introduction 5

Chapter 1 General procedures and methods 10

Chapter 2 Relationship between blood flow and release of metabolites after short-term isometric muscle activity 18

Chapter 3 Blood flow and release of metabolites from exercising muscle after prolonged and short work 21

Chapter 4 Vascular response to adrenergic stimulation during and after prolonged and short work 23

Chapter 5 Vascular response to hyperosmotic stimulation during and after prolonged and short work 27

Chapter 6 Modification of the forearm blood flow response to contralateral isometric handgrip by prolonged and short work 29

Chapter 7 General discussion 32

Conclusions 37

Acknowledgements 38

References 39

Part B

Vascular response to hyperosmotic stimulation during and after prolonged and short work 46

Modification of the forearm blood flow response to contralateral isometric handgrip by prolonged and short work 53

The present thesis is based on the following papers

I Eklund B and L Kaijser Forearm blood flow after isometric contraction at different loads in relation to potentially vasodilating substances
Scand J clin Lab Invest 1974 Accepted for publication

II Kaijser L and B Eklund The effect of noradrenaline and of increased sympathetic activity on the hyperaemia following short and prolonged forearm work
Scand J clin Lab Invest 33 1974 In press

III Eklund B and L Kaijser *The effect of close* arterial infusion of isoproterenol and phentolamine on the blood flow following short-term and prolonged work
Res Commun Chem Pathol Pharmacol 1974 Accepted for publication

IV Eklund B L Kaijser and E Knutsson Blood flow in resting arm and leg during contralateral isometric contraction J Physiol (Lond) 1974 In press

V Eklund B Vascular response to hyperosmotic stimulation during and after prolonged and short work
This supplement Part B

VI Eklund B Modification of the forearm blood flow response to contralateral isometric handgrip by prolonged and short work
This supplement Part B

The papers will be referred to by their Roman numbers

Introduction

PREVALENT OPINIONS CONCERNING BLOOD FLOW REGULATION IN EXERCISING MUSCLE

The increase in blood flow in the exercising skeletal muscle has been studied extensively for many decades and the factors that adapt the muscle blood flow to work intensity and oxygen demand have been subject to much debate The conception that local factors such as chemical or physical changes linked to the metabolism of the muscle produce exercise hyperaemia was suggested already by Gaskell (1880) and that they play the most important role in its maintenance seems now generally accepted

At rest the tone of the precapillary resistance and sphincter sections in the vascular bed of the skeletal muscle is considered to be regulated mainly by an intrinsic myogenic activity facilitated by the distention of contractile elements of the vascular wall (Bayliss 1902, Folkow 1949, Folkow and Öberg 1961) Superimposed on this local regulation of the precapillary vessels is a central vasomotor control which in man is mediated by sympathetic adrenergic fibres (for references see Folkow 1955 and Uvnäs 1960 and 1967) During exercise vasodilating metabolites released from the active muscles overcome to a great extent the myogenic activity as well as the constrictor fibre influence on the precapillary vessels of the muscle while the nervous influence on the postcapillary section is still maintained (Kjellmer 1964 and 1965 a Strandell and Shepherd 1967)

A large number of different substances produced locally during muscle contraction have alone or in combination been suggested as mediators of the increase in muscle blood flow during exercise Histamine was discussed early (Anrep and Barsoum 1935) but its participation in the production of exercise hyperaemia was not confirmed (Fleisch and Weger 1937 Emmelin Kahlson and Wiksell 1941) the same applying to bradykinin (Hilton and Lewis 1958 Kjellmer and Odelram 1965) Lactic and pyruvic acids are produced by the exercising muscle and Molnar et al (1962) investigated the local vascular action of these acids in the canine forelimb Intra-arterial infusion caused a moderate decrease in vascular resistance which was attributed to the concomitant increase in H^+ ions since it was not observed when sodium lactate or pyruvate was infused Furthermore in McArdle's syndrome lactate is not produced by the active muscle

but still muscle blood flow increases markedly during work (Pernow Havel and Jennings 1967) However if not a very potent vasodilator in itself lactate is probably one of the more important substances contributing to the increase in osmolality (Lundvall 1972 Pernow personal comm see below) CO_2 is also produced in the working muscle and the effect of CO_2 has been evaluated by Kontos and co-workers (Kontos and Patterson 1966 Kontos Richardson and Patterson 1967 Kontos 1971) in animal as well as human studies They conclude that local hypercapnia plays only a minor role in the production of hyperaemia in the contracting muscle

Adenosine may according to Berne et al (1971) be involved in the local regulation of skeletal muscle blood flow but is probably more important in the regulation of coronary blood flow (Rubio and Berne 1969) ATP has been identified in the effluent venous blood from ischemic working muscle (Forrester and Lind 1969 Forrester 1972) and since it has a pronounced vasodilator action it is suggested to be involved in the local regulation of flow Hilton (1971) suggested that inorganic phosphate may also be a mediator of exercise hyperaemia Barcroft and co-workers (1971) however were not able to produce any substantial increase in forearm blood flow by intraarterial infusions of inorganic phosphate in amounts that raised the level of phosphate in the effluent venous blood to values far above those seen during exercise

Hypoxia in the wall of the resistance vessels has been suggested by Carrier Walker and Guyton (1964) and Detar and Bohr (1968) as a possible cause of the vasodilatation during exercise but there seems to be little reason to believe that the oxygen tension to which the resistance section of the vessels is exposed decreases sufficiently during work under normal conditions Duling and Berne (1971) have indeed shown that the oxygen tension on the external surface of the artery starts to decrease to a certain extent already between the larger artery and the terminal artery Such an effect must however play a minor role since on the venous side of the muscle vascular bed the PO_2 decreases rather little with an increased work load suggesting only a moderate decrease in PO_2 at tissue level at the same time as blood flow increases severalfold (Wahren 1966 Kaijser 1970)

Potassium ions are released from contracting muscle cells and Kjellmer (1965 b) proposed that the increase in potassium ions in the extracellular fluid can account for a considerable part of the vasodilatation However since plasma concentration of potassium is rapid-

ly normalized after work (Laurell and Pernow 1966) It can thus hardly be responsible for the prolonged hyperaemia following exercise Skinner and Costin (1970 1971) have studied the interaction between oxygen lack and increase in potassium concentration and osmolality and concluded that these factors reinforce one another in lowering the resistance of the canine muscle vascular bed

Most authors agree that probably several factors jointly produce the vasodilatation in the working muscle An attempt to explain the nature of such a joint effect was made by Mellander and co-workers (Mellander et al 1967 Lundvall Mellander and White 1969 Mellander and Lundvall 1971 Lundvall 1972), who suggested hyperosmolality induced by the local formation of several metabolites during exercise to be the essential vasodilating factor in exercise hyperaemia In reviewing the literature Mellander and Johansson (1968) also stated the criteria some of which are given below that should be fulfilled for a given metabolic factor if it is to be accepted as of importance in mediating exercise hyperaemia 1) The substance should belong to the group of agents naturally occurring in muscle tissue It should be demonstrable in the tissue or the effluent venous blood during exercise and the concentration of the substance should bear some quantitative relationship to the degree of vasodilatation 2) Intraarterial administration of the substance in amounts comparable to those released during exercise should in resting muscles give the same magnitude and pattern of vascular response as that of exercise itself 3) The mode of elimination of a dilator factor after infusion should be compatible with the approximately exponential decrease of flow seen in the postexercise period

A major part of the studies on local chemical or physical changes that might influence vascular resistance in working muscles has been performed as animal experiments where muscle contraction has been accomplished by nerve stimulation or on isolated blood vessels which for technical reasons must be fairly large and hence not representative of the terminal arteries involved in resistance regulation (Burton 1954) Electrically induced muscle activity might well differ from muscle activity in man performing exercise with regard to metabolite release Thus forearm muscle activity induced by electrical stimulation seems to result in a greater release of lactate at a given oxygen uptake level than a corresponding voluntarily induced rhythmic dynamic activity (Landin and Wahrén

1968) Studies in man on physiological modes of exercise are relatively few Barcroft (1971) Kontos and co-workers (1966, 1967) and Forrester and Lind (1969) undertook their studies on the human forearm (see above) Lundvall et al (1969) compared the effects of forearm exercise and close intraarterial infusion of hypertonic solutions on blood flow in man in order to establish a quantitative relationship between forearm blood flow and osmolality A hypertonic infusion producing an increase in venous osmolality comparable to that found during exercise did not decrease vascular resistance to the same degree but the authors ascribed the discrepancy to the possibility that muscle activity increased tissue osmolality more than infusion

Most of the studies here referred to have not taken into account the effect of work duration on muscle metabolism and hence its possible influence on flow adaptation by local factors Often the work duration is imprecisely stated but the majority of the studies refer to short periods of muscular activity Ekelund (1967) found that once a relative hemodynamic steady state was established cardiac output remained largely unchanged during bicycle exercise for one hour Jorfeldt (1970) and Wahren and Hagenfeldt (1968) studied the local circulation in the human forearm during dynamic work of the same duration Blood flow was found to increase slightly with the duration of exercise while oxygen uptake remained constant Thus oxygen saturation and tension of the effluent venous blood was increased Furthermore as has been shown earlier (Bang 1936) the lactate concentration in the effluent venous blood decreased with time which is also the case in the muscle tissue (Saltin and Karlsson 1970) and PCO_2 and H^+ were concomitantly lowered Thus some of the chemical changes in the effluent venous blood and muscle tissue are more pronounced during the initial phase of exercise than at the end of a prolonged work period and still blood flow is maintained at an approximately steady level

Aim of the study The relative importance of the different possible mediators of the increased local blood flow during exercise has not been agreed upon Furthermore studies have rarely been performed under conditions of well defined modes and durations of work Therefore the aim of the present investigations was

1 Further to study the relation of blood flow to some of the factors that have been ascribed major importance in the production of exercise hyperaemia in connection with short-term muscle activity

2 To gain more information concerning the importance of different flow promoting factors by studying the relation between flow and metabolite concentration in the effluent venous blood during work of different durations yielding different metabolite release

3 To investigate whether prolonged work induces a change in sensitivity of the muscle vascular bed to vasoactive stimulation which might be of importance for blood flow maintenance since the literature indicates that prolonged work changes the proportion between blood flow and the concentration of some metabolites in the effluent venous blood

The intention was to study the influence of work duration on the relationship between blood flow and metabolites as well as on the sensitivity of the vascular bed both during and shortly after work of different durations One motive for performing studies on vascular reactivity also shortly after work was that studies of the response to vasoactive stimulation require a method that can measure rapid changes in blood flow The only method satisfying this requirement possible to use in human studies seems to be venous occlusion plethysmography a method which however with few exceptions can be applied only on a resting limb

To follow and quantify rapid changes in flow also during work the deep venous O_2 saturation was used as indicator of flow changes

Chapter I

GENERAL PROCEDURES AND METHODS

Subject In all 72 healthy male volunteers of ordinary physical fitness aged 20-54 years (mean age 32) were studied Several of them took part in more than one of the series of experiments

Modes of exercise Exercise was performed with the forearm

Two modes of exercise have been used

1 Sustained isometric handgrip contraction performed on a dynamometer with immovable handles (Flygtekniska Försöksanstalten Stockholm In some experiments a similar dynamometer manufactured by Bofors AB Karlskoga was used) The force developed was measured by a strain gauge and displayed to the subject on an oscilloscope or by the pointer of a galvanometer

2 Rhythmic dynamic forearm work performed on a spring loaded hand ergometer with a contraction path of 40 mm Contraction frequency was 60/min governed by a metronome The load was determined by the length and number of the springs (Kaijser 1970)

The exercise was performed in the recumbent position with the forearm at heart level The isometric contraction was performed with the elbow in a slightly flexed position and the dynamometer in a vertical position the dynamic work with the elbow extended and the dorsal side of the hand facing upwards

Catheterization procedures A brachial artery was catheterized percutaneously for blood pressure recording or the infusion of vasoactive substances Teflon catheters were used with an outer diameter of 1 4 mm and the tip adapted to a guide line with a diameter of 0 9 mm For the infusion of vasoactive substances the catheter was introduced about 10 cm into the brachial artery of the arm to be studied for arterial blood pressure recording the contralateral arm was catheterized

The same type of teflon catheter was used for the catheterization of the deep venous system of the forearm and was introduced into a cubital vein in the distal direction and manipulated into a deep vein so that the tip could not be palpated The distance it had been introduced 4-10 cm was measured and checked again when the catheter was withdrawn after the termination of the

experiment With the catheter in this position it is possible to sample blood draining muscle tissue almost exclusively during exercise and also at rest if the circulation of the hand is occluded (Cooper Edholm and Mottram 1955 Coles et al 1958 Idbohrn and Wahren 1964)

Intraarterial infusions of vasoactive substances were per formed with a motordriven constant speed pump

Forearm blood flow measurement Blood flow in the forearm was measured by venous occlusion plethysmography The plethysmograph consisted of an airfilled rubber cuff according to Dohn (1956) and Graf and Westersten (1959) The plethysmograph cuff was placed around the thickest part of the forearm the enclosed segment being 5 cm long The cuff was inflated to about 40 mm H_2O Volume changes in the forearm were recorded as pressure changes in the plethysmograph and calibrated with a known volume of air The venous occlusion pressure was 50 mm Hg During the recording of inflow curves the circulation of the hand was occluded by a wrist cuff inflated to 240 mm Hg The pressure increase in the plethysmograph following venous occlusion was measured with a pressure transducer (EMT 33 Siemens-Elema) and recorded on an ink jet recorder (Mingograf 34 or 81 Siemens-Elema) Blood flow is expressed in $ml \times min^{-1} \times 100$ ml of tissue^{-1}

Skin and muscle temperatures Skin temperature was measured with a Tele-thermometer (Yellow Springs Instrument Inc) the probe taped to the skin immediately distal to the plethysmograph cuff Muscle temperature was measured with a thermocouple situated at the tip of a thin polyethylene catheter (Ellab Instruments) introduced percutaneously into m brachio-radialis to a depth of 20-25 mm beneath the skin under the plethysmograph cuff

Arterial pressure was measured with a capacitative transducer (EMT 34 or 35 Siemens-Elema) and recorded on a Mingograf 81 (Siemens-Elema) Mean pressure was obtained by electrical integration time constant 1 sec or in the studies presented in Chapter 6 3 sec

Heart rate was obtained from ECG recordings

Blood analyses Blood samples for the determination of O_2 saturation PO_2 PCO_2 and pH were drawn into siliconized glass syringes where the dead space was filled with he-

Calculations Resting or basal forearm blood flow was calculated from at least five inflow curves In the study presented in Chapter 2 (Paper I) the average flow during the first 30 sec period after contraction was calculated for each subject from all inflow curves recorded during this interval and the average flow for the second 30 sec period was calculated similarly At later intervals flow was calculated from four to six successive inflow curves In the series of experiments comparing short and prolonged work the first second and third inflow curves after the 5 and the 55 min work period were averaged separately for the group of subjects At other times inflow curves recorded during a 30 sec period were averaged for the individual subject to represent the flow at that time When flow changes were rapid blood flow values are given for 15 sec intervals and are then calculated from one or two inflow curves Maximal blood flow e g during infusion of vasodilating substances or during contralateral isometric contraction is calculated as the mean of the two steepest successive inflow curves Forearm vascular resistance is calculated as mean arterial pressure divided by forearm blood flow

$$\frac{\text{mm Hg}}{\text{ml} \times \text{min}^{-1} \times 100\ \text{ml}^{-1}}$$

Statistical methods Statistical calculations have been performed according to conventional methods (Snedecor 1959) When not otherwise stated values given in the text and tables are mean values + standard error of the mean for the group studied Significance tests performed on values obtained from a group of subjects at different times have been calculated by paired comparisons

Error of methods The error of the methods used for the various blood analyses are given in Table I In the series of experiments presented in Chapter 5 the osmolality of each blood sample was determined four times The error of the method was then 0 42 per cent of the mean value The error of the forearm blood flow measurements includes biological variations as well as the methodological errors The error of a single determination in per cent of the mean value as estimated from duplicate determinations of resting blood flow was 10 1 per cent (mean 4 33 $\text{ml} \times \text{min}^{-1} \times 100\ \text{ml}^{-1}$ number of duplicate determinations 39) At increased flow rates produced by con-

tinuous prolonged intraarterial infusion of phentolamine the error was 16 7 per cent (mean value 8 43 $ml \times min^{-1} \times 100\ ml^{-1}$ n=32) The reproducibility calculated from blood flow determinations at corresponding intervals after two identical periods of submaximal dynamic forearm work of 15 min duration separated by 90 min rest, was 12 0 per cent of the mean value (mean 25 5 $ml \times min^{-1} \times 100\ ml^{-1}$ n=22)

Definitions In the present publication "short-term work' refers to durations of approximately 5 min and prolonged work to durations of approximately 1 hour

For convenience the term "metabolites' is used to denote all substances released from the active muscle

Table 1 Errors of a single determination as estimated from duplicate determinations

$$E = \sqrt{\frac{\Sigma d^2}{2n}}$$

Variable	Range	Number of duplicate determinat	Mean value	Error	Error in per cent of mean value
O_2 saturation %					
according to *Drabkin* (1950)	10 40	36	30 9	0 34	0 10
	40-90	51	57 1	0 48	0 84
CO-Oximeter(Instr Lab)	30 90	26	59 5	0 86	1 45
PO_2 mm Hg	40-100	25	66 8	2 29	3 43
PCO_2 mm Hg	20-60	25	30 5	0 93	3 08
pH	7 20-7 50	25	7 425	0 009	1 21
Lactate mmol/l	0-2	36	1 37	0 06	4 3
	2-8	32	3 63	0 13	3 8
Pyruvate mmol/l	0-0 10	35	0 072	0 006	8 3
	0 10-0 30	32	0 132	0 005	3 7
Na^+ mEq/l	125-145	20	132 1	0 177	0 13
K^+ mEq/l	2 0-5 0	20	3 81	0 016	0 40
Osmolality mosm/kg	260-310	50	276 7	1 103	0 58

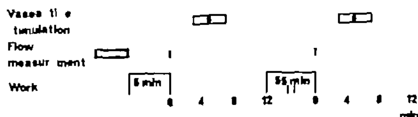


Fig 1 General design of experiments comparing the the effect of vasoactive stimulation on postexercise forearm blood flow after short term (5 min) and prolonged (55 min) work

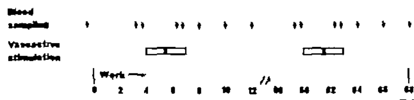


Fig 2 General design of experiments comparing the effect of vasoactive stimulation on deep venous oxygen saturation during short term and prolonged work

Chapter 2

RELATIONSHIP BETWEEN BLOOD FLOW AND RELEASE OF METABOLITES AFTER SHORT-TERM ISOMETRIC MUSCLE ACTIVITY (Paper 1)

As an introduction to the studies of relations between blood flow and metabolites during work of different durations the effect of short-term forearm muscle activity of defined intensities on forearm blood flow and on the concentration of vasoactive substances in the effluent venous blood was investigated Muscle activity was performed as isometric contraction Under these conditions the mechanical effect of the contraction reduces the blood flow substantially (Humphreys and Lind 1963 Edwards Hill and McDonnell 1972) Consequently metabolites can be assumed to accumulate and blood and tissue concentrations of metabolites might tend to equilibrate towards the end of the contraction period

Procedure Seven subjects were studied performing isometric handgrip at 1/3 1/4 and 1/8 MVC for 2 minutes 1/3 MVC is slightly below the load leading to fatigue in 2 minutes Resting and post-contraction forearm blood flow was measured plethysmographically Deep venous blood was sampled at rest at the end of and after the contractions

Results and Discussion Conclusions regarding tissue concentrations of substances drawn from their concentrations in the effluent venous blood from isometrically contracting muscle are uncertain If the blood flow through the muscle is partly hindered the flow is small in relation to the metabolic rate and hence blood and tissue concentrations might tend to equilibrate towards the end of the contraction period In that case the immediate post-contraction blood flow would be better correlated with the blood concentrations of flow-promoting metabolites at the end of the contraction than those during the hyperaemic phase after it If however blood flow through the contracting muscle is completely arrested, there may be an equilibration only of a limited amount of capillary blood and this would attenuate the changes in the venous blood sampled during contraction

The above mentioned circumstances must be considered when conclusions regarding cause and effect are drawn from relationships found between blood flow and

metabolite concentrations in the effluent venous blood of the muscle

Contraction at 1/8 MVC increased forearm blood flow five times the resting level contractions at 1/4 and 1/3 MVC increased the flow further roughly in proportion to the loads The post-contraction blood flows differed significantly between the loads still at three minutes after the contractions

Lactate and pyruvate concentration as well as osmolality increased during contraction to a higher degree with higher load Of these pyruvate concentration decreased immediately after contraction and then again increased thus the changes do not parallel the changes in blood flow Lactate showed a tendency to increase further after contraction and to remain elevated while the blood flow decreased more rapidly This would suggest that lactate concentration is not a specific flow-promoting factor However it may well be one of the more important substances contributing to the increase in osmolality Osmolality increased in five of the subjects at the two lower loads but the change for the group did not reach significance At the highest load it increased by 23 mosm/l Thus the quantitative relationship between peak flow and osmolality was not quite convincing However the decrease in osmolality rather well paralleled the decrease in blood flow indicating that osmolality may be of importance as a flow-promoting factor as suggested by Lundvall Mellander and White 1969 and Lundvall 1972

PCO_2 and acidity increased very little during but markedly after contraction rendering the difference between the loads largest immediately after contraction If the contention is valid that blood flow is arrested by the contraction and the blood sampled after contraction reflects tissue concentrations better than that sampled during it these two factors show a relation to blood flow which might indicate that they contribute to the post-contraction hyperaemia

PO_2 decreased slightly towards the end of the contractions but did not differ between the loads Increased immediately after contractions and furthermore reached the highest level after the heaviest load It therefore seems justifiable to rule out low O_2 tension during or immediately after contraction as a flow-promoting factor under physiological conditions The K^+ concentration increased during contraction but to the same extent at the different loads Furthermore the concentration decreased again immediately after contraction which is in accordance with

the findings of Laurell and Pernow (1966) and thus it does not seem likely that K^+ contributes to the hyperaemia after work

Conclusion Of the different metabolic factors studied osmolality was best correlated with the blood flow pattern after isometric muscle activity Lactate was probably the important substance causing the changes in osmolality Furthermore the data are in accordance with the contention that CO_2 and H^+ to a minor degree might contribute to the vasodilatation

Chapter 3

BLOOD FLOW AND RELEASE OF METABOLITES FROM EXERCISING MUSCLES DURING AND AFTER PROLONGED AND SHORT WORK (Paper II)

The regulation of muscle blood flow during exercise and the possible influence on it of local metabolic factors have mainly been studied in connection with relatively short periods of work without consideration of the effect of work duration on muscle metabolism and thereby eventually on blood flow adaptation The blood concentration of lactate decreases with work duration (Bang 1936) and during forearm work of one hour also the H^+ concentration and PCO_2 of the effluent venous blood is lower than during short work while muscle blood flow is slightly greater (Wahren and Hagenfeldt 1968 Jorfeldt 1970)

In the present series of experiments post-exercise forearm blood flow was recorded after short and prolonged dynamic forearm work in order to gain information regarding possible differences in spontaneous course of flow decrease which might suggest different mechanisms for the maintenance of the hyperaemia The concentration of some conceivably *vasoactive substances was also studied* in the effluent venous blood during and after the short and the prolonged work to establish their relation to the blood flow

Procedure In 14 subjects performing dynamic forearm work at an average load of 7 5 kpm/min for 5 and 55 min forearm blood flow was recorded plethysmographically for 12 min after the work periods without inserted catheters or other interventions In 20 additional subjects a deep vein was catheterized percutaneously for blood sampling at rest as well as during and after the short and the prolonged work Skin and muscle temperatures were recorded in all subjects

Results Forearm blood flow was the same immediately after the short and the prolonged work After the short work flow decreased rapidly at first and then more slowly towards resting level After the prolonged work the decrease during the first minute was even more rapid but from the second minute after work no further decrease took place during the rest of the measurement period blood flow remaining at a level 3 4 times the resting

flow and hence also above the flow at corresponding times after the short work

PO_2 in the deep vein was significantly higher and PCO_2 and H^+concentration lower at the end of the prolonged than at the end of the short work period Also lactate and pyruvate concentrations were lower at the end of the prolonged than the short work During short-term work K^+concentration and osmolality increased slightly but significantly but at the end of prolonged work it had decreased again to the resting level The Na^+concentration was not at all influenced by exercise

Skin and muscle temperatures increased about 1° C during the short and further about 1 5° C during the prolonged work During the 12 min period of blood flow recording after the work periods they remained essentially unchanged

Discussion As evident from the present and earlier studies (Wahren and Hagenfeldt 1968 Jorfeldt 1970) the forearm blood flow during and immediately after prolonged work is slightly larger than or the same as that during and after short-term work However, the venous blood concentration of several substances linked to the metabolism of the muscle such as CO_2 H^+ K^+ and lactate as well as osmolality which were markedly increased during the short work had decreased significantly again at the end of the prolonged work Thus, the local blood flow in working muscle can hardly be regulated by the simple effect of any of these factors mentioned above Theoretically however they may still be of importance either if the sensitivity of the vascular bed to these substances is increased or if the vasoconstrictor effects which they have to counteract are decreased

The time course of disappearance of the hyperaemia differed after prolonged and short work Thus after prolonged work the flow decreased more rapidly at first and then remained unchanged at a level 3-4 times the resting and hence also higher than the flow at corresponding times after short-term work This altered spontaneous rate of flow decrease might indicate that partly different mechanisms maintain the hyperaemia after and possibly also during prolonged work

Tissue temperature seems to be the only variable determined in this study which is higher after prolonged work and might thus be partly responsible for the difference in flow and taken into consideration as a possible factor contributing to maintain muscle blood flow during prolonged work

Chapter 4

VASCULAR RESPONSE TO ADRENERGIC STIMULATION DURING AND AFTER PROLONGED AND SHORT WORK (Papers II and III)

Since the concentration of several vasodilator metabolites in the effluent venous blood from the exercising muscle decreases progressively during prolonged work while the blood flow is maintained at a steady level (Paper II) it must be assumed either that their importance in the regulation of local blood flow is diminished or that either the sensitivity to such metabolites has increased or the vasoconstrictor effects which they have to counteract are decreased by prolonged work In the series of experiments presented in this chapter the possibility of an altered response to adrenergic stimulation induced by prolonged work was investigated The following experimental situations were studied

1 Response to adrenergic vasoconstriction after as well as during short and prolonged work both as the effect of close intraarterial infusion of noradrenaline and as the effect of increased sympathetic nervous activity produced by the application of subatmospheric pressure to the lower part of the body (lower body low pressure LBLP) For the study of vascular response to adrenergic stimulation during exercise changes in deep venous oxygen saturation was used as an indicator of blood flow changes with the assumptions previously discussed (Chapter 1)

2 Modification of the response to infusion of noradrenaline after short and prolonged work by local β-adrenergic blockade

3 Effect on blood flow of β-adrenergic stimulation by close intraarterial infusion of isoproterenol after short and prolonged work

4 Effect of α adrenergic blockade by close intraarterial infusion of phentolamine after short and prolonged work

Procedures In all 61 subjects took part in the investigation performing dynamic forearm work at a load of 7-8 kpm/min In the studies performed during work 0 2 µg noradrenaline per minute was infused intra arterially or LBLP (10-30 mm Hg below atmospheric) was applied for 3 min early during the continuous work period

and again towards the end of it and deep venous blood was sampled as shown in Fig 2 (Chapter 1)

Vasoactive stimulation by LBLP or infusion of drugs shortly after work was performed as shown in Fig 1 (Chapter 1) during the 12 min period of plethysmographic blood flow recording Noradrenaline was infused in a dose of 0 1 µg/min isoproterenol in doses of 0 02-0 08 µg/min and phentolamine in doses of 150-200 µg/min Regional β-adrenergic blockade was obtained by the infusion of 0 5 mg propranolol during the last minute of the work periods (Johnsson 1967)

Results When noradrenaline was infused during the continuou work period the decrease in deep venous O_2 saturation was more pronounced after 60 than after 4 min of forearm work indicating a more pronounced flow reduction (Fig 3) LBLP however induced the ame decrease in deep venous O_2 saturation when applied at 4 and 60 min (Fig 4)

When applied shortly after the work periods noradrenaline and LBLP produced a less pronounced decrease in forearm blood flow after the prolonged than after the short work but this difference is probably accounted for by the difference in spontaneous rate of flow decrease (Paper II) After the prolonged work however noradrenaline infusion was followed by a marked flow increase above the preinfusion level which was seen neither after short work nor on the termination of LBLP in the same situation

Regional β-adrenergic blockade did not significantly influence the flow decrease during noradrenaline infusion but abolished the increase in flow above preinfusion level on the termination of infusion after prolonged work

Isoproterenol infusion after the short work increased the forearm blood flow to a value more than twice the preinfusion flow The flow decreased again but remained above the preinfusion value during the whole infusion period After prolonged work isoproterenol increased flow by 50 per cent which is significantly less than after the short work

Phentolamine increased forearm blood flow only slightly by about 15 per cent when infused after the short work After the prolonged work the increase was significantly larger being nearly 60 per cent

Discussion The findings have provided some indications that prolonged work might increase the response of the forearm vascular bed to α- as well as β-adrenergic effects

of infused noradrenaline In contrast no difference in response to increased sympathetic nerve activity could be discerned The observation that the large increase in blood flow on the termination of noradrenaline infusion after prolonged work could be abolished by β-adrenergic blockade initiated the study of the response to β-adrenergic stimulation by isoproterenol The flow increase in response to infusion of isoproterenol however was less pronounced after prolonged work and thus not in accordance with the hypothesis that prolonged work might induce a greater sensitivity to β-adrenergic stimulation However there might be differences in response to a bloodborne β-adrenergic stimulating agent and the transmitter released from the nerve terminal The response to an α-adrenergic blocking agent phentolamine was more marked after prolonged work This finding might imply that α adrenergic stimulation or the sensitivity to such stimulation, is more pronounced after prolonged work However the latter explanation seems to be excluded by the earlier described observations An additional explanation would be that the sensitivity to the non-adrenergic vasodilator effect which has been ascribed to phentolamine (Taylor et al 1965 a b and c) is more pronounced after prolonged work

Conclusion The quantitative response to increased adrenergic nervous vasoconstrictor stimulation seems not to be altered to a significant degree by prolonged work which however induces a qualitatively altered response to circulating noradrenaline which might be due to a more long-lasting β-adrenergic stimulation Phentolamine caused a greater blood flow increase after prolonged than after short work probably due to an increased sensitivity to the direct vasodilator action of the drug

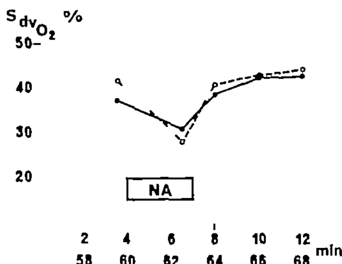


Figure 3 Effect of infusion of noradrenaline on deep venous oxygen saturation during short (filled circles full line) and prolonged (unfilled circles broken line) Mean values for 10 subjects

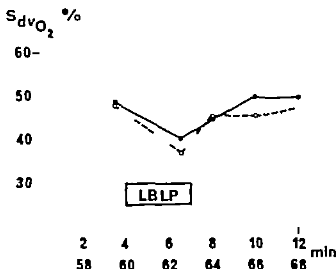


Figure 4 Effect of lower body low pressure (LBLP) on deep venous oxygen saturation during short and prolonged work Symbols as in Fig 3 Mean values for 6 subjects

Chapter 5

VASCULAR RESPONSE TO HYPEROSMOTIC STIMULATION DURING AND AFTER PROLONGED AND SHORT WORK (Paper V)

During prolonged forearm work the local hyperosmolality which has been suggested as an important mediator of muscle hyperaemia gradually decreases again as evidenced by the osmolality of the effluent venous blood and still the blood flow is maintained (Paper II) If osmolality is an important factor operating also during prolonged work the vascular response to changes in osmolality must be assumed to become more pronounced with the duration of work To test this hypothesis the effect of an increase in blood osmolality on forearm blood flow in connection with short and prolonged work was compared Infusions of a hyperosmotic solution was made both 1/ during and 2/ shortly after short and prolonged work

Procedures 1 Eight subjects performed a continuous forearm work at an average load of 8 kpm/min for 68 min A 20 per cent solution of glucose was infused through a catheter in the brachial artery at a rate of 5 ml/min for 3 min from the 4th and the 60th minute Deep venous blood for the determination of O_2 saturation and osmolality was sampled before at the end of and again after the infusion periods (Fig 2 Chapter 1) With the assumptions discussed in Chapter 1 changes in deep venous O_2 saturation were taken to indicate changes in forearm muscle blood flow

2 Ten subjects performed forearm work at an average work load of 8 kpm/min for 5 and 55 min Forearm blood flow was recorded plethysmographically for 12 min after each work period and 20 per cent glucose was infused at a rate of 3 ml/min for 4 min starting at 3 min after the work periods (Fig 1 Chapter 1)

Results After 3 min work deep venous osmolality had increased by about 7 mosm/kg and after 60 min work it had decreased again to a value not significantly above the resting venous level The infusion of hyperosmotic glucose during work increased deep venous osmolality to the same extent 8 mosm/kg at 4 and 60 min The infusion at 4 min was accompanied by a 23 per cent increase in deep venous O_2 saturation indicating an increase in forearm blood flow After 60 min work however the infusion of glucose did not affect the O_2 saturation

After short and prolonged work the infusion of hyperosmotic glucose was accompanied by a similar flow increase being slightly more than 100 per cent in both situations In the individual subjects blood flow showed marked oscillations during the infusions

Discussion It might be argued that the extent to which adequate mixing between blood and the infusate has been obtained is uncertain According to Wahren (1968) a higher infusion rate 30 ml/min is required during light work for complete mixing

However it does not seem likely that the degree of mixing would differ systematically between infusions performed in connection with short and prolonged work since the blood flow at the start of infusion is similar in the two situations Furthermore in the experiments where the hyperosmotic solution is infused during work conclusions regarding changes in forearm blood flow are drawn from the O_2 saturation of the venous blood draining the working muscle the estimation of the extent to which the infusion affects blood osmolality is made on blood from the same vein

Thus in addition to the fact that deep venous osmolality decreases with the duration of work the results show that the sensitivity to hyperosmolality is not increased but rather reduced during prolonged work Consequently this factor cannot be ascribed a general role as a mediator of exercise hyperaemia although it might well be of importance during the early phase of muscular work

Chapter 6

MODIFICATION OF THE FOREARM BLOOD FLOW RESPONSE TO CONTRALATERAL ISOMETRIC HANDGRIP BY SHORT AND PROLONGED WORK (Papers IV and VI)

Exercise changes the vasomotor nervous outflow and provided this change is generalized the effect of altered vasomotor influence might be studied in a limb not taking part in the exercise where the response is not modified by local metabolic and mechanical factors According to Lind et al (1964) the vascular resistance of the resting arm increases during contralateral isometric contraction In some of their subjects however a decrease was found but was attributed to inadvertent muscle activation Bevegård and Shepherd (1966) found an increase in vascular resistance in the resting arm also during dynamic leg work but this was preceded by an initial decrease in resistance

In the present study the pattern of blood flow response in the resting arm to contralateral isometric contraction and the neurogenic mechanisms behind it were evaluated The stimulus of contralateral handgrip was then utilized to affect blood flow in a forearm resting after prolonged and short work respectively

Procedure In all 29 subjects were studied Isometric contraction was performed as a handgrip at 1/3 MVC or as a dorsiflexion of the foot at 1/2 MVC ECG and arterial pressure were recorded continuously Blood flow in the resting arm was measured plethysmographically To ascertain that inadvertent muscle activation did not influence blood flow in the resting arm EMG was recorded in some experiments The contralateral isometric contraction was repeated after regional β- and α-adrenergic blockade produced by close intraarterial infusion of either 0 5 mg propranolol given slowly in a single dose or 200 μg phentolamine/min as a continuous infusion In the last series forearm blood flow was followed plethysmographically for 17 min after short and prolonged forearm work at a load of 8 kpm/min and contralateral isometric handgrip was applied at 3 8 and 13 min In connection with the second of these contractions deep venous blood from the resting forearm was also sampled

Results Arterial mean pressure increased linearly during the isometric contraction Blood flow in the resting forearm increased rapidly to a maximal value of two and a

half times the precontraction flow Towards the end of contraction it decreased again but still remained twice the precontraction value thus vascular resistance remained below that before contralateral handgrip The increase in flow was not due to inadvertent activation, since maximal flow was recorded early during contraction when electromyographic activity was only rarely recorded Dorsiflexion of the foot caused the same pattern of blood flow response in the resting forearm as did contralateral handgrip

Local β-adrenergic blockade reduced the flow increase during contralateral handgrip substantially During α-adrenergic blockade the flow increase continued throughout the contraction period and no tendency to flow decrease was seen towards the end of the contraction

The blood flow in the previously working forearm was increased by contralateral handgrip to a greater extent after prolonged than short-term work The comparison was rendered somewhat difficult by the fact that precontraction flow often differed after short and prolonged work However, the difference in response remained even if pairs of contractions were compared where precontraction flow was similar

Discussion Contralateral handgrip induced a rapid increase in the resting forearm blood flow appearing before any substantial increase in arterial pressure signifying a decrease in vascular resistance Thus the flow increase must to a great extent be the result of neurogenic vasodilator mechanisms Since it was considerably reduced after regional β-adrenergic blockade the nature of the mechanism is evidently β-receptor activation The relative increase in resistance towards the end of the contralateral contraction which was abolished by α-adrenergic blockade might be interpreted as the result of a gradual change to predominantly α-adrenergic effects The neurogenic vasomotor effects seem to be generalized since the same pattern of flow response was induced by leg and hand contraction However in addition to the mentioned neurogenic effects distension by the increased arterial pressure might contribute to the flow increase and vascular smooth muscle reaction to the distending force might be involved in the terminal relative flow decrease

The more marked flow increase produced by contralateral handgrip during the hyperaemia after prolonged than after short work might be interpreted as the result

of increased β-adrenergic effects especially since the difference is most pronounced during the early phase The extent to which such an interpretation is valid is somewhat unclear in the light of the earlier described finding of a less pronounced effect of isoproterenol on forearm blood flow after prolonged work Thus tentatively, a preliminary interpretation would be that prolonged work also produces a diminished tendency to respond with an increase in myogenic tone to the intravascular pressure increase during isometric contraction

Chapter 7

GENERAL DISCUSSION

Factors which might mediate the dilatation of the muscle vascular bed during exercise and adapt blood flow to the increased oxygen demand have been the subject of numerous investigations Local chemical and physical changes caused by the increase in metabolic rate are generally believed to produce the flow increase (for review see Mellander and Johansson 1968 Ross 1971) and several substances released from the working muscle have vasodilator effects The increased concentration of lactate H^+ and K^+ the increase in PCO_2 and decrease in PO_2 as well as the increased osmolality may all contribute but their relative importance is not established The duration of work has been shown to affect the release of several of the above mentioned substances such as lactate H^+ and CO_2 (Wahren and Hagenfeldt 1968 Jorfeldt 1970) without affecting exercise hyperaemia to a major degree and work duration can be expected to influence the local conditions in the working muscle also in other respects Thus it seems reasonable to assume that the release also of other vasodilator factors may change with the duration of work or that the vascular response to a given vasodilator stimulus is altered

The aim of the present study was to elucidate the relative importance of a number of locally active substances by relating the muscle blood flow to their release as reflected by their concentrations in the effluent venous blood in connection with exercise of well defined modes intensities and durations Special attention was given to possible changes in the relation of blood flow to the release of vasoactive ubstances during prolonged work and to possible changes in the response of the muscle vascular bed to vasoactive stimuli

Exercise performed with the forearm was used since the active muscle mass is small and the amount of meta bolites produced is small enough not to affect their con centration in the arterial blood Furthermore the musculature is usually supplied by one artery the brachial artery permitting the infusion of substances for the study of their effects on forearm blood flow and due to the small tissue mass in amounts which do not produce systemic effects Furthermore the deep venous system of the forearm drains almost exclusively muscle tissue thus permitting the sampling of blood for analyses of substances released from the active muscle (Coles et al 1958 Idbohrn and Wahren 1964)

The forearm consists of muscle tissue to about 60 per cent (Abramson and Ferris 1940 Cooper, Edholm and Mottram 1955) Thus under conditions of increased muscle blood flow such as postexercise hyperaemia blood flow measured plethysmographically probably to a great extent represents muscle blood flow, since skin blood flow has been shown to be unchanged by dynamic forearm work of moderate duration (Wahren 1966) Furthermore the direct effects on skin vascular resistance of changes in skin temperature, within the range observed during prolonged work in the present study are apparently small (Catchpole and Jepson 1955 Spealman 1945 Paaske Hovind and Sejrsen 1973)

In studies on the blood flow response to intra-arterial infusion of different vasoactive substances the extent to which adequate mixing is accomplished must be taken into consideration According to Wahren (1968) an infusion rate of 30 ml/min is required during moderate work in order to obtain complete mixing In view of the possibility that the infusion as such then may affect the blood flow and render to response to different stimuli difficult to evaluate lower rates of infusion have been used and thus the extent to which mixing has been obtained is uncertain However conclusions are drawn from paired comparisons between effects of infusions in connection with short and prolonged work at similar levels of blood flow and hence there is no reason to believe that the degree of mixing would differ systematically

The decline in blood flow was initially somewhat faster after prolonged than after short work but levelled off after 2-3 min at 3 times the resting value whereas after short work blood flow continued to decrease gradually At 3 min the blood flow in most instances was at a similar level after the short and the prolonged work and this point of time was therefore chosen for the start of infusion of vasoactive substances or the application of LBLP However because of the difference in course of spontaneous flow decrease it was still difficult to drawn firm conclusions regarding differences in response to vasoactive stimulation after short and prolonged work Consequently only substantial differences in response or responses appearing very early during the period of stimulation or in the opposite direction of the difference in spontaneous flow decrease after short and prolonged work can be taken as evidence of an altered vascular response induced by prolonged work

The concentration of vasoactive substances in effluent venous blood produced by short-term muscle activity was first studied using isometric contractions at varying fractions of MVC Osmolality showed the closest parallelity to postexercise blood flow both with regard to maximal values and the time course for the return towards resting level which is in accordance with the contention brought forward by Mellander and co-workers (Lundvall Mellander and White 1969 Mellander and Lundvall 1971 Lundvall 1972) and local osmolality may under these conditions play an important role However from the present findings it cannot be excluded that both lactate in addition to its contribution to osmolality pH and PCO_2 may also be considered as possible factors contributing to the postcontraction hyperaemia *Deep venous* K^+ *concentration and* PO_2 *did not* follow the flow changes either with respect to magnitude or time course and thus it seems reasonable to conclude that they do not contribute significantly to the hyperaemia after the isometric contraction

In accordance with the findings by Wahren and Hagenfeldt (1968) and Jorfeldt (1970) blood flow immediately after prolonged dynamic forearm work was the same or slightly higher than after short work In addition to a decrease in deep venous lactate CO_2 and H^+ during prolonged work that has been demonstrated earlier (Bang 1936 Wahren and Hagenfeldt 1968 Jorfeldt 1970) osmolality and K^+ concentration which were significantly raised during short work did not differ from resting levels at the end of prolonged work It can therefore be concluded that during prolonged work muscle blood flow could not have been maintained by these factors unless either the response of the vascular bed to them was increased or the neurogenic or intrinsic myogenic vasomotor effects on vascular resistance had changed

Of these alternatives the possibility of an altered response to factors related to the increased metabolism was first taken into consideration Since Mellander and co-workers (Mellander et al 1967 Lundvall Mellander and White 1969 Jonsson 1970 Lundvall 1972) on the basis of intensive studies on the vasodilating effect of hyperosmolality put forward the hypothesis of osmolality as an essential physiological regulator of regional blood flow it seemed important to study the effect of increased osmolality on forearm blood flow during exercise of varying duration An increase in local osmolality elicited by the infusion of a hyperosmotic glucose solution did raise deep venous oxygen saturation indicating an increase in blood flow during short but not prolonged work This finding implies a diminished response

of the muscle vascular bed to hyperosmotic stimulation during prolonged work while at the same time the local hyperosmolality induced by the muscle activity is less pronounced Consequently it appears reasonable to conclude that while local hyperosmolality may be an important factor contributing to the increase in muscle blood flow during work of short duration it cannot be of significance in the regulation of blood flow during prolonged work

Since the results did not support the conception that the response to the vasodilator action of hyperosmolality increases with prolonged work the possibility that prolonged work alters the response to adrenergic stimulation was also tested

The response to increased sympathetic nerve activity induced by lower body low pressure was probably not different in connection with prolonged compared to short work However the difference in response after short and prolonged work is somewhat difficult to interpret because of the difference in spontaneous rate of flow decrease On the other hand when noradrenaline was infused intraarterially during work a more marked flow reduction was found in connection with prolonged than with short work After prolonged work a qualitative change in response was observed to the effect that the infusion of noradrenaline was followed by a substantial increase in flow which was not seen after the short work and which could be abolished by β-adrenergic blockade

It is conceivable that the effect of circulating noradrenaline on the vascular smooth muscle is changed by prolonged work increasing both the α- and the β-adrenergic stimulating properties The qualitatively altered response might be of importance for the maintenance of blood flow during prolonged work although it can certainly not fully explain the unaltered exercise hyperemia

These findings prompted a further study of the effect of β-adrenergic stimulation and of α-adrenergic blockade on the hyperaemia after prolonged and short work respectively The infusion of isoproterenol could not confirm an increased response to β-adrenergic stimulation at least not to the type of stimulation used The response to phentolamine might indicate an increased adrenergic vasoconstrictor stimulation or an increased sensitivity to it after prolonged work but earlier studies seem to give no support to such an interpretation Since other effects of the drug have been described (Taylor et al 1965 a b and c) an additional explanation seems to be that prolonged work

induces a more pronounced response to its direct vasodilating effects

Finally the response of the muscle vascular bed to vasoactive stimulation in connection with prolonged and short work was studied also as the response to a contralateral isometric handgrip

An isometric contraction performed as a handgrip could be shown to increase blood flow in the contralateral resting forearm on an average 2 5 times the resting flow indicating a decreased vascular resistance A further analysis suggested that β-adrenergic effects are partly responsible for this flow increase especially during the early phase of contraction while the continuously rising arterial pressure might become more important towards the end of the contraction period when also a probable increase in sympathetic vasomotor activity might become more vasoconstrictive in effect as indicated by the relative increase in resistance towards the end of the contraction

Contralateral handgrip contraction increased blood flow in the previously working forearm significantly more when performed during the postexercise hyperaemia after prolonged than after short work Since the flow increase produced by contralateral handgrip apparently is the result of a complex interaction between neurogenic sympathetic effects arterial pressure increase and a myogenic reaction of the vascular smooth muscle induced by the increase in intravascular pressure the implication of a larger and more rapid flow increase after prolonged work is not obvious

The far more rapid onset of the flow increase after prolonged work suggests that after all a higher sensitivity to β-adrenergic stimulation is induced but since the earlier studies gave little support to the contention of an increased sensitivity to β-adrenergic stimulation a preliminary interpretation of the results may be that prolonged work in addition lowers the reactivity of the vascular smooth muscle to the increase in arterial pressure A lowered intrinsic myogenic tone might then be a factor contributing to the maintenance of flow during prolonged work

CONCLUSIONS

1 The local increase in osmolality might contribute significantly to the blood flow increase in exercising muscle during work of short duration

2 During prolonged work osmolality decreases towards the resting level and in addition the response of the vascular bed to an increase in blood osmolality is reduced Consequently hyperosmolality cannot be ascribed a general role as mediator of exercise hyperaemia

3 Prolonged work induces quantitative and qualitative changes in response to adrenergic stimulation but not of a magnitude that explains the maintenance of blood flow

4 The considerable flow increase induced by phentolamine which was found after prolonged work is therefore to a large extent interpreted as a more marked response to a nonadrenergic smooth muscle relaxing property of the compound and thus the vascular response to non-neurogenic stimuli seems to be altered by prolonged work

5 A strong isometric contraction induces an increase in blood flow in the resting arm to a great extent due to β-adrenergic effect during the initial phase The pattern and magnitude of the response is not dependent on the muscle group used indicating a generalized increase in adrenergic vasomotor activity during contraction

6 Contralateral isometric contraction performed after short and prolonged work increases the blood flow in the resting arm more markedly when in its hyperaemic phase after prolonged than after short work As differences in adrenergic reactivity between the two situations seem moderate the finding might imply a change in myogenic reactivity of the vascular smooth muscle contributing to the maintenance of blood flow in exercising muscle during prolonged work

ACKNOWLEDGEMENTS

To Professor Torgny Sjöstrand who initiated this study I wish to express my sincere thanks for many valuable suggestions

To Professor Bengt Pernow I am most grateful for the interest he has shown and for important advice and criticism

To Assistant Professor Lennart Kaijser who has been my teacher and has shown a never failing interest I wish to express my sincere gratitude for many stimulating and instructive discussions

Warm thanks goes to Mrs Gertrud Grünfarb for most valuable advice and secreterial help in the preparation of this thesis and also to Miss Anita Lundmark and Mrs Birgitta Edholm who greatly helped me with manuscript

To all members of the laboratory staff I am sincerely grateful for their kind help and interest

The study has been supported by grants from the Karolinska Institute and from Svenska Sällskapet för Medicinsk Forskning

REFERENCES

1 Abramson D I & Ferris E B Responses of blood vessels in the resting hand and forearm to various stimuli Amer Heart J 19 541 1940

2 Anrep G V & Barsoum, G S Appearance of histamine in the venous blood during muscular contraction J Physiol (Lond) 85 409 1935

3 Bang O The lactate content of the blood during and after muscular exercise in man Skand Arch Physiol 74 51 1936

4 Barcroft H Foley T H & McSwiney R R Experiments on the liberation of phosphate from the muscles of the human forearm during vigorous exercise and on the action of sodium phosphate on forearm muscle blood vessels J Physiol (Lond) 213 411 1971

5 Bayliss W M On the local reactions of the arterial wall to changes of internal pressure J Physiol (Lond) 28 220 1902

6 Berne R M Rubio R Dobson J G & Curnish R R Adenosine and adenine nucleotides as possible mediators of cardiac and skeletal muscle blood flow regulation Circulat Res 28 suppl I 115 1971

7 Bevegård B S & Sheperd J T Reaction in man of resistance and capacity vessels in forearm and hand to leg exercise J appl Physiol 21 123 1966

8 Burton A C Relation of structure to function of the tissues of the wall of blood vessels Physiol Revs 34 619 1954

9 Bücher T Czok R Lamprecht W & Latzko E Purncat In Methoden der Enzymatischen Analyse pp 253-259 Verlag Chemie Weinheim 1962

10 Carrier O Walker J R & Guyton A C Role of oxygen in autoregulation of blood flow in isolated vessels Amer J Physiol 206 951 1964

11 Catchpole B N & Jepson R P Hand and finger blood flow Clin Sci 14 109 1955

12 Coles D R Cooper K E Mottram R F & Occleshaw J V The source of blood samples withdrawn from deep forearm veins via catheters passed upstream from the median cubital vein J Physiol (Lond) 142 323 1958

13 Cooper K.E Edholm O G & Mottram R F The blood flow in skin and muscle of the human forearm J Physiol (Lond) 128 258 1955

14 Detar R & Bohr D F Oxygen and vascular smooth muscle contraction Amer J Physiol 214 241, 1968

15 Dohn K Plethysmographs usable during functional states recording volume changes in ml per 100 ml of extremity Rep Steno Hosp 6 147 1956

16 Drabkin D L Measurement of O_2-saturation of blood by direct spectrophotometric determination In Methods in Medical Research 2 159 1950 Year Book Publishers Chicago

17 Duling B R & Berne R M Oxygen and the local regulation of blood flow Possible significance of longitudinal gradients in arterial blood oxygen tension In Local regulation of blood flow Circulat Res 28 suppl I 65 1971

18 Edwards R T H Hill D K & McDonnel M Myothermal and intramuscular pressure measurements during isometric contractions of the human quadriceps muscle J Physiol (Lond) 224 58 1972

19 Ekelund L -G Circulatory and respiratory adaptation during prolonged exercise Acta physiol scand 70 Suppl 292 1967

20 Emmelin N Kahlson G & Wicksell F Blood histamine in reactive hyperaemia and in muscle tetanus Acta physiol scand 2 110 1941

21 Fleisch A & Weger P Über das Auftreten von gefässerweiternden Sub tanzen in venösen Blut Pflügers Arch ges Physiol 239 354 1937

22 Folkow B Intravascular pressure as a factor regulating the tone of the small vessels Acta physiol scand 17 289 1949

23 Folkow B Nervous control of the blood vessels Physiol Rev 35 629 1955

24 Folkow B & Öberg B Autoregulation and basal tone in consecutive vascular sections of the skeletal muscles in reserpine treated cats Acta physiol scand 53 105 1961

25 Forrester, T An estimate of adenosine triphosphate release into the venous effluent from exercising human forearm muscle J Physiol (Lond) 224 611 1972

26 Gaskell W H On the tonicity of the heart and blood vessels J Physiol (Lond) 3 48 1880

27 Graf K & Westersten A Untersuchungen über Eigenschaften und Verwendungsmöglichkeiten eines flexiblen Extremitätenplethysmografen Acta physiol scand 46 1 1959

28 Hilton, S M A new candidate for mediator of functional vasodilation in skeletal muscle In Regulation of Blood Flow Circulat Res 28 Suppl I 70 1971

29 Hilton S M & Lewis G P Vasodilation in the tongue and its relationship to plasmakinin formation J Physiol (Lond) 144 532 1958

30 Hohorst H -J L(+)Lactat Bestimmung mit Lactat Dehydrogenase und DNP In Methoden der Enzymatischen Analyse Verlag Chemie Weinheim 266 1962

31 Holmgren A & Pernow B Spectrophotometric measurement of oxygen saturation of blood in the determination of cardiac output A comparison with the van Slyke method Scand J clin Lab Invest 11 143, 1959

32 Humphreys P W & Lind A R The blood flow through active and inactive muscles of the forearm during sustained handgrip contractions J Physiol (Lond) 166 120, 1963

33 Idbohrn, H & Wahren J The origin of blood withdrawn from deep forearm veins during rhythmic exercise Acta physiol scand 61 301 1964

34 Johnsson G The effects of intraarterially administered propranolol and H 56/28 on blood flow in the forearm a comparative study of two β-adrenergic receptor antagonists Acta pharmacol et toxicol 25 Suppl 2 63 1967

35 Jonsson O Extracellular osmolality and vascular smooth muscle activity Acta physiol scand Suppl 359 1970

36 Jorfeldt L Metabolism of L(+)-lactate in human skeletal muscle during exercise Acta physiol scand 78 Suppl 338 1970

37 Kaijser L Limiting factors for aerobic muscle performance Acta physiol scand 79 Suppl 346 1970

38 Kjellmer I The effect of exercise on the vascular bed of skeletal muscle Acta physiol scand 62 18 1964

39 Kjellmer I On the competition between metabolic vaso dilation and neurogenic vasoconstriction in skeletal muscle Acta physiol scand 63 450 1965a

40 Kjellmer I The potassium ion as a vasodilator during muscular exercise Acta physiol scand 63 460 1965b

41 Kjellmer I & Odelram H The effect of some physiological vasodilators on the vascular bed of skeletal muscle Acta physiol scand 63 94 1965

42 Kontos H A Role of hypercapnic acidosis in the local regulation of blood flow in skeletal muscle In Local Regulation of Blood Flow Circulat Res 28 Suppl I 98 1971

43 Kontos H A & Pattersson J L Carbon dioxide as a major factor in the production of reactive hyperemia in the human forearm Clin Sci 27 143 1964

44 Kontos H A Richardson D W & Pattersson J L Blood flow and metabolism of forearm muscle in man at rest and during sustained contraction Amer J Physiol 211 868 1966

45 Kontos H A Richardson D W & Pattersson J L Effects of hypercapnia on human forearm blood vessels Amer J Physiol 212 1070 1967

46 Landin S & Wahren J Blood flow oxygen uptake and lactate production in the forearm during exercise induced by median nerve stimulation Acta physiol cand 75 82 1969

47 Laurell H & Pernow B Effect of exercise on plasma potassium in man Acta physiol scand 66 241 1966

48 Lind A R Taylor S H Humphreys P W Kennelly B M & Donald K W The circulatory effects of sustained voluntary muscle contraction Clin Sci 27 229 1964

49 Lundholm L Mohme-Lundholm E & Vamos N Lactic acid assay with L(+) lactic acid-dehydrogenase from rabbit muscle Acta physiol scand 58 243 1963

50 Lundvall J Tissue hyperosmolality as a mediator of vasodilation and transcapillary fluid flux in exercising skeletal muscle Acta physiol scand 85 Suppl 379 1972

51 Lundvall J Mellander S & White T Hyperosmolality and vasodilation in human skeletal muscle Acta physiol scand 77 224 1969

52 Mellander S & Johansson B Control of resistance exchange and capacitance functions in the peripheral circulation Pharmacol Rev 20 117 1968

53 Mellander S & Lundvall J Role of tissue hyperosmolality in exercise hyperemia In Local Regulation of Blood Flow Circulat Res 28 Suppl I 39 1971

54 Mellander S Johansson B Gray S Jonsson O Lundvall J & Ljung B The effects of hyperosmolality on intact and isolated vascular smooth muscle Possible role in exercise hyperemia Angiologica 4 310 1967

55 Molnar J I Scott J B Frolich E D & Haddy F J Local effects of various anions and H^+ on dog limb and coronary vascular resistances Amer J Physiol 203 125 1962

56 Paaske W P Hovind H & Sejrsen P Influence of therapeutic ultrasonic irradiation on blood flow in human cutaneous subcutaneous and muscular tissues Scand J clin Lab Invest 31 389 1973

57 Pernow B Havel R J & Jennings D B The second wind phenomenon in McArdles syndrome Acta med scand Suppl 472 294 1967

58 Rubio R & Berne R M Release of adenosine by the normal myocardium in dogs and its relationship to the regulation of coronary resistance Circulat Res 25 407 1969

59 Saltin B & Karlsson J Muscle glycogen utilization during work of different intensities In Muscle Metabolism during Exercise Ed B Pernow & B Saltin p 289 1970

60 Segal S Blair A E & Wyngaarden An enzymatic spectrophotometric method for the determination of pyruvic acid in blood J Lab clin Med 48 137 1956

61 Skinner N S & Costin J C Interactions of vaso-active substances in exercise hyperemia O_2 K^+ and osmolality Amer J Physiol 219 1386 1970

62 Skinner N S & Costin J C Interactions between oxygen potassium and osmolality in regulation of skeletal muscle blood flow In Local Regulation of *Blood Flow Circulat Res 28 Suppl I 73 1971*

63 Snedecor G W & Cochran W G Statistical Methods The Iowa State University Press Iowa 1967

64 Spealman, C R Effect of ambient air temperature and of hand temperature on blood flow in hands Amer J Physiol 145 218 1945

65 Stainsby W N & Barclay J K Effect of infusions of osmotically active substances on muscle blood flow and systemic blood pressure In Local Regulation of Blood Flow Circulat Res 28 33 1970

66 Strandell T & Sheperd J T The effects in humans of increased sympathetic activity on the blood flow to active muscles Acta med scand 11 Suppl 472 146 1967

67 Taylor S H Sutherland G R MacKenzie G J Staunton H P & Donald K W The circulatory effects of intravenous phentolamine in man Circulation 31 741 1965a

68 *Taylor S H Sutherland G R MacKenzie G J* Staunton H P & Donald K W The effect of adrenergic blockade on the circulatory response to exercise in man Clin Sci 28 117 1965b

69 Taylor S H Sutherland G R MacKenzie G J Staunton H P & Donald K W The circulatory effects of phentolamine in man with particular respect to changes in forearm blood flow Clin Sci 28 265 1965c

70 Uvnäs B Central cardiovascular control In Handbook of Physiology Neurophysiology Amer Physiol Soc 2 1131 sect 1 1960

71 Uvnäs B Cholinergic vasodilator innervation to *skeletal muscles Circulat Res 20 21 Suppl I* 83 1967

72 Wahren J Quantitative aspects of blood flow and oxygen uptake in the human forearm during rhythmic exercise Acta physiol scand 67 Suppl 269 1966

73 Wahren J & Hagenfeldt L Human forearm muscle metabolism during exercise I Circulatory adaptation to prolonged forearm exercise Scand J clin Lab Invest 21 257 1968

VASCULAR RESPONSE TO HYPEROSMOTIC STIMULATION DURING AND AFTER PROLONGED AND SHORT WORK

The increase in blood flow through exercising muscle has been ascribed to the action of several metabolically linked vasoactive substances (Mellander and Johansson 1968 Haddy and Scott 1968 Ross 1971) but the relative importance of the different factors has not been assessed A mechanism by which several substances might act together to produce vasodilatation was offered by Mellander and co-workers (Mellander et al 1967 Lundvall Mellander and White 1969 Mellander and Lundvall 1971 Lundvall 1972) who suggested that the local hyperosmolality produced by the formation of osmotically active metabolites during exercise plays an important role in the regulation of muscle blood flow during exercise

However as shown earlier (Kaijser and Eklund 1974) the osmolality of the effluent venous blood that increases during the early phase of muscular work is significantly reduced during prolonged work At the same time blood flow is unchanged or slightly increased (Wahren and Hagenfeldt 1968 Jorfeldt 1970) If osmolality is an important general factor in the regulation of the local blood flow in exercising muscle operating also during prolonged work then the vascular sensitivity to changes in osmolality must be assumed to have increased with the duration of work

To test this possibility the effect on forearm blood flow of increased osmolality accomplished by close intraarterial infusion of a hypertonic glucose solution was studied during and after short and prolonged forearm work

I Effect of close intraarterial infusion of hypertonic glucose on forearm blood flow during short and prolonged work

Subjects Eight subjects aged 25-36 years were studied

Procedure Percutaneous catheters were introduced into the brachial artery and a deep vein of the working arm The subjects performed dynamic forearm work at a load of 8 kpm/min (Kaijser 1970) A 20 per cent solution of glucose was infused into the brachial artery at a rate of 5 ml/min for 3 minutes from the fourth and the sixtieth minute of a continuous work period of 68 min duration Arterial blood for the determination of osmo lality was sampled at rest and before the infusion periods Deep venous blood for the determination of osmolality and oxygen saturation was sampled at rest and in duplicate before and again at the end of the infusion periods Single samples were withdrawn at 1 3 and 5 minutes after the infusion periods Based on the assumption that the oxygen consumption remained constant during a continuous work at a fixed intensity and that it was not to a signi ficant degree influenced by glucose infusion changes in deep venous oxygen saturation were taken as evidence of changes in forearm muscle blood flow

Results Arterial osmolality was the same at rest and befc e the infusions at 4 and 60 min of the work period At rest deep vein osmolality did not differ from that of arterial blood After 3 min work the venous osmolality was significantly increased above the arterial and the resting venous level by about 7 mosm/kg ($p < 0\ 001$) After 60 min work it had decreased again to a value not significantly above the arterial and the resting venous level

The infusion of hypertonic glucose increased deep vein osmolality to the same extent about 8 mosm/kg at 4 min and 60 min of the work period (Fig 1)

At rest the deep vein O_2 saturation was $51\ 9 \pm 3\ 8$ per cent After 3 min work it was $32\ 6 \pm 2\ 0$ per cent and the increase in osmolality produced by the infusion of 20 per cent glucose was accompanied by a 23 per cent increase ($p < 0\ 01$) in O_2 saturation to a mean value of $38\ 0 \pm 1\ 3$ per cent (Fig 1) indicating an increase in forearm blood flow Three minutes after the infusion it had returned to the preinfusion level After 60 min work however the infusion of glucose producing the same in crease in osmolality did not affect the oxygen saturation of the effluent venous blood

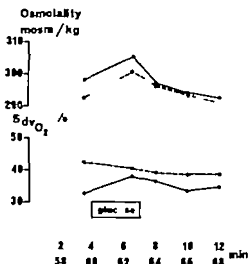


Figure 1 Effect of intraarterial infusion of a 20 per cent glucose solution on deep venous osmolality and oxygen saturation during short (•-•) and prolonged (o-o) work Mean values for 8 subjects

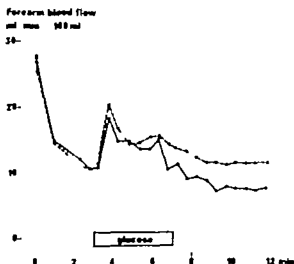


Figure 2 Effect of intraarterial infusion of a 20 per cent glucose solution on forearm blood flow after 5 (•-•) and 55 (o-o) min work Mean values for 10 subjects

References

HADDY F J and J B SCOTT Metabolically linked vaso-active chemicals in local regulation of blood flow Physiol Rev 1968 48 688-707

JORFELDT L Metabolism of L$^{(+)}$-lactate in human skeletal muscle during exercise Acta physiol scand 1970 78 Suppl 338

KAIJSER L Limiting factors for aerobic muscle performance Acta physiol scand 1970 79 Suppl 346

KAIJSER L and B EKLUND The effect of noradrenaline and of increased sympathetic activity on the hyperaemia following short and prolonged forearm work Scand J clin Lab Invest 1974 33 00-00

LUNDVALL J S MELLANDER and T WHITE Hyperosmolality and vasodilatation in human skeletal muscle Acta physiol scand 1969 77 224-233

LUNDVALL J Tissue hyperosmolality as a mediator of vasodilatation and transcapillary fluid flux in exercising skeletal muscle Acta physiol scand 1972 85 Suppl 379

MELLANDER S and B JOHANSSON Control of resistance exchange and capacitance functions in the peripheral circulation Pharmacol Rev 1968 20 117-196

MELLANDER S B JOHANSSON S GRAY O JONSSON J LUNDVALL and B LJUNG The effects of hyperosmolarity on intact and isolated vascular smooth muscle Possible role in exercise hyperemia Angiologica 1967 4 310-322

MELLANDER S and J LUNDVALL Role of tissue hyperosmolality in exercise hyperemia Circulat Res 1971 28 Suppl 1 39 45

ROSS G The regional circulation Ann Rev Physiol 1971 33 445-478

STAINSBY W N and J K BARCLAY Effect of infusions of osmotically active substances on muscle blood flow systemic blood pressure In Local regulation of bl flow Circulat Res 1970 28 33-38

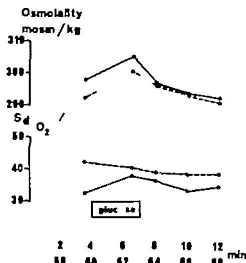


Figure 1 Effect of intraarterial infusion of a 20 per cent glucose solution on deep venous osmolality and oxygen saturation during short (●-●) and prolonged (o-o) work Mean values for 8 subjects

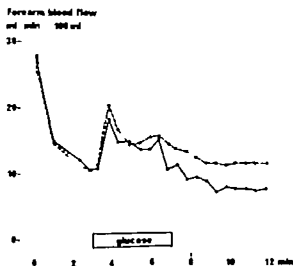


Figure 2 Effect of intraarterial infusion of a 20 per cent glucose solution on forearm blood flow after 5 (●-●) and 55 (o-o) min work Mean values for 10 subjects

MODIFICATION OF THE FOREARM BLOOD FLOW RESPONSE TO CONTRALATERAL ISOMETRIC HANDGRIP BY PROLONGED AND SHORT WORK

Exercise affects the vasomotor nervous outflow and the effect of the thereby altered vasomotor influence can be studied in a limb not taking part in the exercise where the response is not modified by local metabolic and mechanical factors Lind et al (1964) studying the cardiovascular effects of an isometric contraction found that the blood flow in the resting forearm usually remained unchanged in spite of a pronounced increase in arterial pressure thus indicating an increased forearm vascular resistance In some of their subjects however an increase in the resting forearm blood flow was recorded but this was suggested to be the consequence of inadvertent muscle activation Eklund Kaijser and Knutsson (1974) however found a substantial decrease in the vascular resistance of the resting forearm during contralateral isometric handgrip appearing rapidly during the contraction Studies with EMG recordings showed that it could not be due to inadvertent muscle activation The fact that blood flow increased rapidly at a point of time when arterial pressure was only moderately increased suggested the contribution of neurogenic mechanisms apart from a passive distention produced by the increased intravascular pressure The aim of the present series of investigations was to study how the flow response to contralateral isometric handgrip is modified by a previous prolonged and short forearm work since earlier studies indicated a changed reactivity of the vascular bed after prolonged work (Kaijser and Eklund 1974 Eklund and Kaijser 1974) To facilitate an evaluation regarding the extent to which adrenergic neurogenic mechanisms contribute to the blood flow response the contralateral handgrip was also performed with the resting forearm under the influence of selective α- and β adrenergic blockade

1 Effect of regional β- and α-adrenergic blockade on the forearm blood flow response to contralateral isometric handgrip

<u>Subjects</u> Six subjects aged 22-42 years were studied

<u>Procedure</u> For the infusion of drugs and for pressure recording a catheter was introduced percutaneously into the brachial artery of the resting arm Isometric contraction with the contralateral arm was performed as a handgrip with 1/3 of maximal voluntary capacity (MVC) for 2 minutes MVC ranged from 45-60 kp Blood flow in the resting forearm was recorded plethysmographically for 2 minutes before contraction during contraction and for 2 minutes after contraction ECG and arterial pressure were recorded continuously

The subjects performed three contractions with blood flow recording in the resting arm without adrenergic blockade three contractions after regional β-adrenergic blockade of the resting arm and three contractions after regional α-adrenergic blockade To obtain β-adrenergic blockade 0 5 mg propranolol was infused slowly during 1 minute through the arterial catheter in the resting arm 4 minutes before the start of each contraction (Johnsson 1967) α-adrenergic blockade was produced by the continuous infusion of phentolamine through the arterial catheter in a dose of 200 μg/ml and minute starting 4 minutes before each contraction Arterial mean pressure was recorded simultaneously

<u>Results</u> In the control situation the flow increase in the resting arm during contralateral isometric contraction was of the same order of magnitude as in the previous study (Eklund Kaij er and Knutsson 1974) and so were the changes in heart rate and arterial pressure Heart rate and arterial pressure changes were not significantly affected by the drugs given

After regional β-adrenergic blockade the forearm blood flow was 3 8$\pm$0 4 ml×min^{-1}×100 ml^{-1} which is not significantly different from the control situation The flow increase produced by contralateral isometric handgrip was markedly less pronounced than without blockade ($p < 0$ 05) especially during the early part of the contraction (Fig 1)

α-adrenergic blockade increased forearm blood flow before contraction to 7 6$\pm$1 3 ml×min^{-1}×100 ml^{-1} The magnitude of increase in flow in the resting forearm during

contralateral handgrip was not significantly different from that in the control situation However the forearm blood flow increased continuously towards the termination of contralateral handgrip with no tendency to a terminal flow reduction (Fig 1)

Discussion β-adrenergic blockade by propranolol did not significantly alter the basal forearm blood flow but reduced the flow increase caused by isometric contralateral handgrip especially during the first minute of contraction The findings thus support the conception that β-adrenergic receptor stimulation is important for the flow increase If the assumption is made that β-receptors were completely blocked about 50 per cent of the flow increase is due to β-adrenergic effects

During α-adrenergic blockade by phentolamine the basal flow was doubled This makes a direct comparison with the flow response in the control situation difficult However the flow increase in the resting arm continued throughout the contraction period This finding may indicate that the relative increase in resistance towards the end of contraction seen in the control situation could have been the result of a gradual change to predominantly α-adrenergic effects presumably as a result of an increased sympathetic nerve activity which in turn probably was induced by the increased effort to hold the contraction (Clarke et al 1957 Freyschuss 1970)

2 Effect of short and prolonged work on the forearm blood flow response to contralateral isometric handgrip

Subjects Twelve subjects aged 20-36 years were studied

Procedure For arterial pressure recording a brachial artery was catheterized percutaneously For the determination of oxygen saturation a catheter was also introduced into a deep vein of that forearm which was to perform the short and the prolonged dynamic work

Forearm blood flow was recorded plethysmographically Before the start of dynamic forearm work two recordings of the blood flow reaction in the resting arm during contralateral isometric handgrip were undertaken The subject then performed dynamic forearm work (Kaijser 1970) at a load of 8 kpm/min for 5 minutes Forearm blood flow recording was started as soon as possible after work and the flow recording was continued for 17 minutes During this period contralateral isometric handgrip was performed three times namely at 3 8 and 13 minutes after work and held for 2 minutes each time The subject then worked for 55 minutes and the prolonged work was followed by flow recording and contralateral handgrip as after the short work

Arterial blood pressure and ECG were recorded continuously Deep venous blood for the determination of oxygen saturation was sampled in connection with the second contralateral isometric handgrip before dynamic forearm work and after the 5 and 55 min work periods Sampling was performed immediately prior to and at 30 60 90 and 120 seconds of contralateral handgrip and again at 30 and 90 seconds after it Deep venous oxygen saturation was determined also at the end of the 5 and 55 min dynamic work

Results The effect of a contralateral handgrip on the blood flow in the resting forearm did not differ from that earlier recorded (Eklund Kaijser and Knutsson 1974) nor did the effect on heart rate and arterial mean pressure (Fig 2) Deep venous oxygen saturation being 53 0$\pm$3 8 per cent before contraction increased to a maximal value of 65 9$\pm$3 3 per cent during the contralateral handgrip ($p < 0$ 001) (Fig 2)

Heart rate and arterial pressure changes in the individual subjects were well reproducible when handgrip was repeated after short and prolonged work The blood flow response to contralateral isometric handgrip during the hyperaemic phase following short and prolonged work

is shown in Fig 3 The absolute increase in forearm blood flow was significantly larger after the prolonged than the short work ($p < 0\ 001$ for paired comparison of all contractions at corresponding times after work) The difference is most evident during the first 45 seconds of contralateral handgrip

Deep venous oxygen saturation was 36 5±2 3 per cent at the end of the short work At the end of the prolonged work it was slightly higher being 42 9±3 0 per cent ($p < 0\ 01$) During the second contralateral isometric contraction performed after the short and the prolonged work it increased significantly as shown in Fig 4 ($p < 0\ 001$ in both situations)

As earlier described (Kaijser and Eklund 1974) the blood flow tended to be greater after the prolonged than the short work and hence the percentual increases did not differ significantly To facilitate comparison of the response after short and prolonged work those pairs of contractions were analyzed separately where forearm blood flow did not differ more than 25 per cent before the start of the contralateral handgrips after short and prolonged work Twelve pairs fulfilled this criterium Of these six were from the first handgrip after the exercise periods four from the second and two from the third (Fig 5) In these cases both the relative and the absolute increase in blood flow were significantly greater after the prolonged work ($p < 0\ 05$ and $< 0\ 001$ respectively)

In order to examine if the magnitude of the blood flow response to contralateral handgrip was dependent upon the precontraction flow level the maximal absolute as well as the maximal relative flow increase during contraction was plotted against the mean flow during the 30 seconds immediately prior to contraction (Figs 6 and 7) Data are presented for all contractions those performed at rest as well as during the hyperaemic phase after the short and the prolonged work The absolute increase was not dependent upon the precontraction blood flow level

Discussion The fact that the forearm blood flow remains at a higher level after prolonged work renders a comparison of the blood flow response to contralateral isometric handgrip after short and prolonged work difficult However the increase in forearm blood flow seems not to be dependent upon the blood flow level before contralateral contraction During the first contral handgrip in the hyperaemic after the short w

the blood flow increase is superimposed on the spontaneous flow decrease (Kaijser and Eklund 1974) and thus the flow increase produced by contralateral contraction will be somewhat underestimated After the prolonged work however the flow increase is significantly greater already after 45 seconds of the contraction when this source of error should be of little importance Thus it seems reasonable to conclude that there is a difference in blood flow reaction of the resting arm to contralateral isometric handgrip induced by a preceding prolonged dynamic work This conclusion is further supported by the finding that the increase in deep venous saturation is the same after the short and the prolonged work Assuming a constant arterial oxygen saturation and no difference in oxygen consumption of the forearm muscles at the second contralateral handgrip the calculated a-v O_2 difference shows a larger percentage decrease after the prolonged work Furthermore the increase in deep venous oxygen saturation supports the contention that the flow increase to a great extent occurs in the forearm muscles

The blood flow increase in the resting forearm during contralateral isometric contraction is probably the result of a complex interaction between mechanical distension (Folkow and Löfving 1956) changes in the intrinsic myogenic tone as a response to the increase in pressure (Bayliss 1902 Folkow 1949) and sympathetic neurogenic effects as earlier discussed (Eklund Kaijser and Knutsson 1974) In the present study it has been shown that of these factors β-adrenergic effects were of importance especially during the early phase of contralateral contraction However as earlier experiments (Eklund and Kaijser 1974) have demonstrated prolonged work does not increase the sensitivity to β-adrenergic stimulation, i e the response to isoproterenol was less pronounced after prolonged work The more marked flow increase in the resting arm produced by contralateral handgrip in the hyperaemic phase after prolonged work migh then tentatively be interpreted to a substantial degree to result from a reduced tendency to respond with an increase in myogenic tone to intravascular pressure increase This altered mode of response might contribute to maintain flow during prolonged work

Conclusion A contralateral isometric handgrip that has been shown to increase the resting forearm blood flow does so also in a resting forearm in the hyperaemic phas after work The induced flow increase is more pronounced

after a prolonged than after a short work β-adrenergic effects contribute to the flow increase However since earlier studies have not shown any major change in β-adrenergic sensitivity after prolonged work the more pronounced flow response to contralateral handgrip after prolonged work is probably to a great extent due to a reduced myogenic response

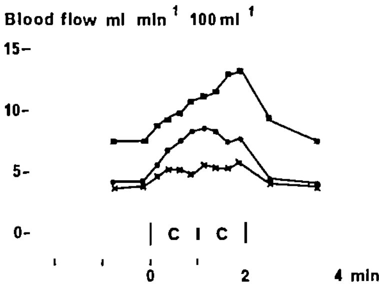


Figure 1 Effect of local β- and α-adrenergic blockade on the increase in the resting forearm during contralateral isometric contraction ●-● control x-x after β-adrenergic blockade ■ ■ after α-adrenergic blockade Mean values for 6 subjects

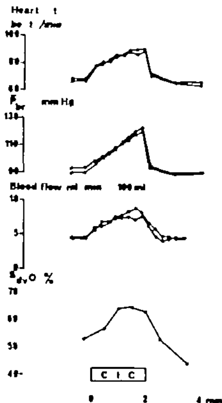


Figure 2 Effect of contralateral isometric contraction on heart rate blood pressure blood flow and deep venous oxygen saturation in the resting forearm Mean values for 12 subjects

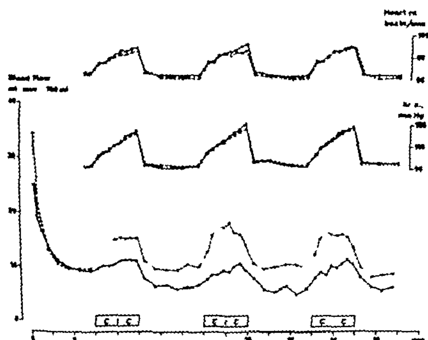


Figure 3 Effect of contralateral isometric contraction on heart rate and arterial pressure and on forearm blood flow after 5 min (• •) and 55 min (o o) forearm work Mean values for 12 subjects

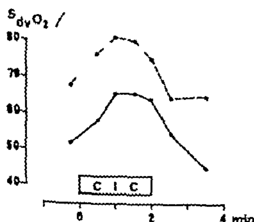


Figure 4 Effect of the second contralateral isometric contraction on deep venous oxygen saturation during the hyperaemia after 5 (• •) and 55 min (o o) forearm work Mean values for 12 subjects

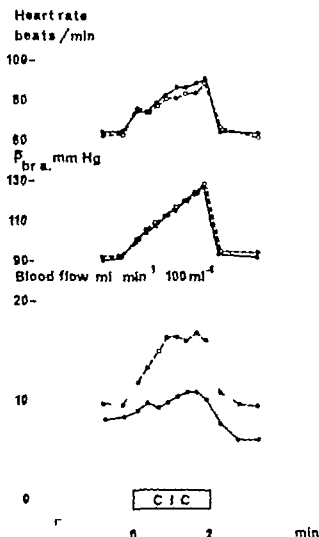


Figure 5 Effect of contralateral isometric contraction on heart rate and arterial pressure and forearm blood flow after short (●-●) and prolonged (o-o) work Pairs of contractions where forearm blood flow before the start of the contralateral isometric contraction did not differ more than 25 per cent Mean values for 12 pairs

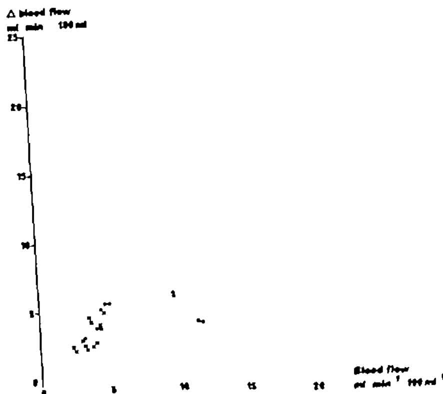


Figure 6 Maximal absolute flow increase during contralateral isometric contraction in relation to precontraction flow Values for contractions performed at rest (y) after 5 min work (•) and after 55 min work (o)

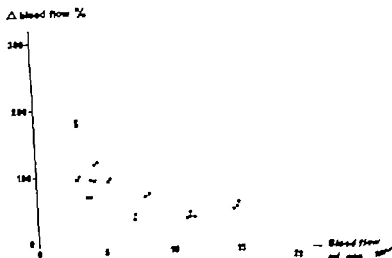


Figure 7 Maximal percentage flow increase during contralateral isometric contraction in relation to precontraction flow Symbols as in Fig 6

References

BAYLISS W M On the local reactions of the arterial wall to changes of internal pressure J Physiol (Lond) 1902 28 220-231

CLARKE R S J R F HELLON and A R LIND The duration of sustained contractions of the human forearm at different muscle temperatures J Physiol (Lond) 1958 143 454-473

EKLUND B and L KAIJSER The effect of close arterial infusion of isoproterenol and phentolamine on the blood flow following short-term and prolonged work Res Commun Chem Pathol Pharmacol 1974 In press

EKLUND B L KAIJSER and E KNUTSSON Blood flow in resting arm and leg during contralateral isometric contraction J Physiol (Lond) 1974 In press

FREYSCHUSS U Cardiovascular adjustment to somatomotor activation Acta physiol scand 1970 Suppl 342

FOLKOW B Intravascular pressure as a factor regulating the tone of the small vessels Acta physiol scand 1949 17 289-310

FOLKOW B and B LÖFVING The distensibility of the systemic resistance blood vessels Acta physiol scand 1956 38 37-52

JOHNSSON G The effect of intra-arterially administered propranolol and H 56/28[2] on blood flow in the forearm a comparative study of two β-adrenergic receptor anta gonists Acta pharmacol & toxicol 1967 25 63-74

KAIJSER L Limiting factors for aerobic muscle perfor mance Acta physiol scand 1970 Suppl 346

KAIJSER L and B EKLUND The effect of noradrenaline and of increased sympathetic activity on the hyperemia following short and prolonged work Scand J clin Lab Invest 1974 33 00-00

LIND A R S H TAYLOR P W HUMPHREYS B M KENNELLY and K W DONALD The circulatory effects of sustained voluntary muscle contraction Clin Sci 1964 27 229-244

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 412

THE ORGANIZATION OF THE ASCENDING CATECHOLAMINE NEURON SYSTEMS IN THE RAT BRAIN

AS REVEALED BY THE GLYOXYLIC ACID FLUORESCENCE METHOD

BY

OLLE LINDVALL and ANDERS BJÖRKLUND

LUND 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 412

FROM THE DEPARTMENT OF HISTOLOGY
UNIVERSITY OF LUND LUND SWEDEN

THE ORGANIZATION OF THE ASCEN
CATECHOLAMINE NEURON SYST
IN THE RAT BRAIN

AS REVEALED BY THE GLYOXYLIC ACID FLUORESCENCE METHOD

BY

OLLE LINDVALL and ANDERS BJÖRKLUND

LUND 1974

INTRODUCTION

The noradrenaline (NA)- and dopamine (DA)-containing cell groups in the lower brain stem are known to give rise to widespread ascending projections to many diencephalic and telencephalic regions. They can be regarded as highly interesting components of the ascending pathways from the brain stem reticular formation Although much information has been gained about the projections of these catecholamine (CA) neuron systems, above all through the extensive work of Fuxe, Ungerstedt and collaborators [1, 19, 21, 64, 65, 81] and of Maeda and collaborators [41, 42] the knowledge of the organization of the CA systems is still far from complete This can at least partly be referred to the difficulties inherent in the methodology employed, which usually requires the use of lesions of the axons in order to increase the CA content of their non terminal parts to levels sufficient for their visualization by the Falck Hillarp formaldehyde fluorescence method.

The introduction of the glyoxylic acid (GA) fluorescence method [7, 35, 36] provided a new and very useful tool for sensitive and detailed morphological studies of central NA and DA neurons. From our preliminary observations with this technique in the rat brain it became evident that the GA method reveals a considerably richer and much more informative picture of the CA fibre systems than was previously possible and that it allows the direct tracing — in the untreated intact animal — of the CA axons from their cell bodies of origin up to their ultimate ramifications. Furthermore several so far unknown CA-containing axon systems were detected in the GA treated specimens. In the present study the GA method was employed for a description of the principal organization of the ascending CA pathways from the brain stem in the rat. This account is primarily concerned with the identity composition, course, and primary branching patterns of the rostrally projecting CA fibre tracts, and attempts have been made to relate these systems to established neuroanatomical nomenclature.

MATERIAL AND METHODS

Brain tissue was studied from about 300 female Sprague-Dawley rats (180—250 g body weight). The *fluorescence histochemical analysis* was performed with the GA method according to Lindvall and Björklund [36] Briefly the procedure was: brain pieces from GA-perfused rats were sectioned at about 30 μ (frontal sagittal and horizontal planes) in a Vibratome Instrument (Oxford Instruments, San Mateo Calif., USA). The sections were immersed in a GA solution, dried, and treated with GA vapour or heated at +100 C according to the procedure described by Lindvall and Björklund [35] As controls for the specificity of the studied fluorescence non-GA treated sections from fresh non-perfused brains were used.

The *microspectrofluorometric analysis* was performed according to Björklund, Ehinger and Falck [4] and Björklund, Falck and Owman [5] with a Leitz microspectrofluorometer The spectra thus obtained were compared with those of the

GA-induced fluorophores of authentic CAs in histochemical models and in tissues[35]

The nomenclature used follows essentially that of König and Klippel [33] and Zeman and Innes [66]: these sources were also used in the preparation of some of the drawings.

RESULTS

In the GA method the intraneuronal primary CAs, NA and DA yield a UV stable, blue or greenish-blue fluorescence (depending on the filter setting), making fluorescent the entire axons, the cell bodies, and variable portions of the dendrites of the CA-containing neurons. However the morphology of the perikarya is often more or less distorted. The identity of the studied structures as CA-containing was established according to the specificity criteria given by Lindvall and Björklund [35]. Randomly selected structures in various parts of the brain, having the characteristic greenish-blue GA-induced fluorescence were analysed microspectrofluorometrically and the spectral characteristics were found to be identical to those of the GA-induced fluorophores of DA and NA (cf. ref. 35). The secondary CA, adrenaline, yields very low fluorescence in the GA method [35]: possible adrenaline-containing structures can therefore be expected not to show up in the GA-treated sections. With the GA-perfusion and immersion procedure employed in the present study indolamine (e. g. serotonin)-containing structures showed a very weak and variable fluorescence also after MAO-inhibition. Their fluorescence was brownish-yellow and faded fairly rapidly upon UV irradiation thus being easily distinguishable from the fluorescence of the CA-containing structures.

The ascending CA fibre systems originating in mesencephalic, pontine, and medullary DA- and NA-containing cell groups and projecting through the lower brain stem towards diencephalic and telencephalic regions were traced in serial sagittal, frontal, and horizontal sections. The following description refers to these ascending fibre pathways. Thus, branching patterns and local projections within the lower brain stem — as well as projections towards cerebellum and the spinal cord — are dealt with only to a very limited extent.

The ascending CA axons originating in pontine and medullary cell groups were confined to two major fibre systems. One system ascends along the tegmental fascicles of Forel [20]. It is partly loosely arranged and occupies almost the entire cross-section of the pontine and mesencephalic tegmentum, in a position corresponding to the *central tegmental tract (CTT)* as defined by e. g. Bürgi and Bucher [13], Nauta and Kuypers [52] and Wünscher *et al.* [67] with classical neuroanatomical techniques (cf. Discussion). In the dorsomedial part of the pontine and mesencephalic CTT the CA fibres are more densely aggregated and typically arranged in fascicles. These aggregated fascicles are distinguished as a separate bundle within the CTT: it is identical with the dorsal CA bundle of Ungerstedt [64]. In the following it is referred to as the *dorsal tegmental bundle (DTB)*.

The second major fibre system is a prominent periventricular-periaqueductal

emergence from the locus coeruleus however the further course of these crossing fibres was not determined.

Along its course through mesencephalon the DTB gives off fibres in several *directions. Within and just rostral to the decussation of the superior cerebellar* peduncles, fibres leave the DTB rostroventrally (and somewhat laterally) along the so-called tegmental radiations, which will be described in more detail below These fibres were seen to consist both of axons deviating from the bundle, and of collateral branches from axons continuing rostrally Laterally many single fibres leave the DTB (both as deviating fibres and as collaterals) towards the dorsal tegmental regions and the geniculate bodies. Dorsally two branches of the DTB project into the posterior and anterior colliculi, respectively The branch to the posterior colliculus — consisting primarily of collateral fibres — leaves the DTB in a dorsal and slightly caudal direction at the level of the decussation of the superior cerebellar peduncles. The branch to the anterior colliculus deviates from the DTB somewhat further rostrally in a rostrodorsal direction. This branch consists of fascicles of DTB fibres deviating from the lateral part of the main bundle

At the meso-diencephalic junction the DTB is situated ventrolateral to the periventricular gray of the third ventricle, close to Darkschewitsch s and Cajal's nuclei. From here, the main bundle bends ventrally and somewhat laterally to pass in between the fasciculus retroflexus and the medial lemniscus, and then just lateral to the mamillothalamic tract. Here CA cells belonging to the caudal thalamic A 11 group were observed to project axons into the DTB Gradually along its course through the middle hypothalamus, the DTB merges with the MFB system. *As described in more detail by Lindvall et al* [37] *the DTB gives off* several branches in the caudal diencephalon that contribute importantly to the adrenergic innervation of the thalamus (see Fig. 1). Thus, ventral to the posterior commissure fibres leave the bundle dorsally to cross the midline in the commissure. Some of these fibres run out into the contralateral pretectal region and others probably join the contralateral DTB When passing between the fasciculus retroflexus and the medial lemniscus, one component leaves the bundle dorsorostrally along the surface of the fasciculus retroflexus and another bends laterally to fan out on the dorsal surface of the medial lemniscus. These branches of the DTB project into the medial and midline regions and the ventrobasal complex, respectively of the thalamus. Further rostrally a fourth branch is seen to deviate from the DTB along (within and along the surface of) the mamillothalamic tract to reach the anterior thalamic nuclei. At the level of the subthalamic nucleus — shortly before the bundle joins the MFB — some DTB fibres bend laterally and somewhat dorsally to run intermingled with fibres of the nigrostriatal DA system (see below) into the internal capsule. Here some of them were seen to take a rostroventral course towards the CA fibre systems in the supraoptic decussations and the ansa lenticularis (dealt with in more detail below). Others could possibly reach the cortex through the neostriatum. The further rostral course of the DTB within the MFB system is described below in connexion with the MFB

B THE CENTRAL TEGMENTAL TRACT

The fibres of this CA system could, in sagittal sections, be traced along the surfaces of the myelinated tegmental fascicles from the medulla oblongata into the internal capsule and the supraoptic decussations (see Fig 2). The tract is richest in CA axons, and has its maximum extension, in pons and the caudal mesencephalon, here the adrenergic fibres occupy practically the entire cross section of the pontine and mesencephalic tegmentum (Figs. 2 and 6). Part of this CTT system could be followed caudally into the medulla oblongata. Here most of the CTT fibres are aggregated into a dense bundle situated just ventrolateral to the hypoglossal nucleus and ventral to the dorsal vagal nucleus. Going rostrally this bundle passes between the root fibres of the facial nerve and just ventral to the genu of the facial nerve (Fig. 3 A) and when reaching the level of the locus coeruleus the bundle deviates slightly ventrally into a position in the pontine and mesencephalic CTT immediately ventrolateral to the DTB (Fig. 2). Ventral and medial to this dense medullary CTT bundle there occurs, in medulla oblongata and pons, a system of loosely arranged longitudinally running CA fibres, many of which have a peculiar smooth (nonvaricose) appearance. These fibres are probably packed two or more close together into single fibre aggregates (see Fig. 3 B). In the sagittal sections they could be followed from the region of the caudal half of the inferior olive to the rostral pons, here they may turn medially to run further rostrally along the raphe. The origins and terminations of these fibres were not determined.

The medullary CTT bundle (MB in Figs. 1 and 2) consists of both ascending and descending fibres. A major descending component comprised axons that could be traced back to the caudal part of the principal locus coeruleus. They run first ventrally and then bend sharply caudally through and around the ventral part of the genu of the facial nerve caudally they were traced to the level of the nucleus of the solitary tract. To judge from the observations of Loizou [39] after lesions in the locus coeruleus, this descending locus bundle might partly give rise to the extensive CA terminal systems present in this nucleus and in the adjoining dorsal vagal nucleus and the commissural nucleus.

The ascending medullary CA axons originate in the CA cell group in the lateral reticular nucleus (group A 1 according to the nomenclature of Dahlström and Fuxe [19]) and probably also in the cell group (A 2) situated mainly within the dorsal vagal nucleus and the commissural nucleus. The axons from the cell bodies in the lateral reticular nucleus reach the CTT bundle along a straight dorsomedial course. The A 2 cells situated dorsomedially and rather close to the bundle project axons ventrolaterally into the bundle. Many of the A 1 and A 2 axons were seen in sagittal sections to branch in a T-shaped manner in the bundle to give rise to an ascending and a descending collateral.

In the pons the number of CA axons and the size of the CA fibre tract increase considerably owing to the inflow of pontine fibre systems into the CTT In the region of the pontine CA cell groups, the CA fibre arrangements are highly complex, as several abundant axonal systems partly intermingle with

each other and with areas of dense fluorescent terminal networks. In this intricate situation the elucidation of the branching patterns, terminations, and decussations of the fibre systems within the pons must await further experimental work. However the *principal* features of the ascending systems were revealed already in the GA treated material of the intact rat.

In sagittal and frontal sections, three major inflows to the CTT were observed from the pontine cell groups. The most caudal inflow is from the CA cell group (A 5) situated lateral to the superior olivary nucleus, close to the outgoing fibres of the facial nerve. These axons run along the root of the facial nerve and join the ascending medullary CTT bundle. The second, massive inflow of fibres was from the region of the subcoeruleus CA cell bodies (A 7) along the ventral and medial aspects of the superior cerebellar peduncle (Fig. 2). It seems quite probable that also the more dorsally situated CA cells belonging to the A 4 group could contribute to this inflow. As illustrated in Fig. 2 the subcoeruleus fibres run in their initial course in a rostroventral direction and soon turn rostrally to pass through and ventral to the decussation of the superior cerebellar peduncles as a broad loosely arranged system in the CTT. Here, they join fibres coming from the laterally situated subcoeruleus cell bodies, and possibly also fibres coming from the contralateral side. In a cross section at this level (Fig. 6) the subcoeruleus fibres fill practically the entire tegmentum ventrolateral to the DTB. The fibres running in the dorsomedial part of this subcoeruleus fibre system intermingle with the medullary CTT bundle (described above) which runs through the decussation of the superior cerebellar peduncles in a position ventrolateral to the DTB (Fig. 2).

The third inflow of fibres to the CTT was from the principal locus coeruleus. These axons were of the same morphological type as those ascending in the DTB and in fact the projection of axons from the locus coeruleus into the CTT appeared in the sagittal sections as a more loosely arranged ventral extension of the DTB as illustrated in Fig. 1. The locus fibres ascending in the CTT are thus primarily located in the region ventral to the DTB and medial to the medullary and pontine bundles, and they were seen to run straight through the tegmental radiations, along a course parallel to the DTB into the MFB system.

In sagittal and frontal sections large and almost vertically oriented flows of fibres were observed (Fig. 6) interconnecting the CTT with the periventricular gray of the fourth ventricle including the locus coeruleus region. Many of these fibres are attached to the CTT fibres in a T-shaped bifurcation. Their direction is uncertain but it seems most probable (cf. refs. 16 and 64) that these vertically arranged fibre systems largely represent collateral branches from the CTT

Fig. 2. Semidiagrammatic representation of the ascending and descending CA fibre systems in the central tegmental tract (CTT) and its caudal extension, the medullary CA bundle (MB). Rostrally part of the nigrostriatal pathway is represented in its extension through the internal capsule (CI) and the globus pallidus. Composite drawing of different sagittal pla es. Note in particular that the mesencephalic and pontine parts of the drawing illustrat plan situated about 0.5 mm more medial than the rest. For abbreviations, see Index

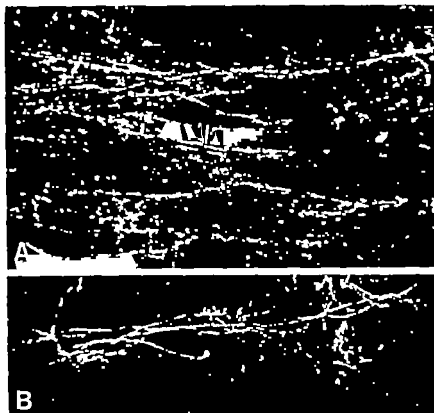


Fig 3 A Ascending and descending fibres in the medullary bundle of the central tegmental tract at the level of the genu of the facial nerve. (X 320) B. CA fibres of the smooth, nonvaricose appearance in the rostral medulla oblongata, running medially in the central tegmental tract system. (X 240).

running dorsally to give rise to the extensive terminal systems distributed in the locus coeruleus region and in the rostral part of the dorsal raphe nucleus and the adjoining ventrolateral central gray (see below) It is also conceivable that they could contribute axons to the dorsal periventricular system.

The pontine and medullary fibres in the CTT run through and ventral to the decussation of the superior cerebellar peduncles, and on its rostral side the dorsal parts of the system turn rostroventrally to fan out along the so-called tegmental radiations (described below) (Figs. 2 and 7). As illustrated schematically in Fig. 16 these CTT fibres follow the radial course of the tegmental radiations for varying distances and a significant portion of the system leaves the radiations after only a short distance to resume their longitudinal course, now in more ventral and lateral positions. Other CTT fibres follow the tegmental radiations down into the ventrolateral tegmentum. Here they join the most ventral CTT fibres (passing ventral to the decussation of the superior cerebellar peduncles) to contribute to the formation of the MFB system (see below). Thus, rostral to the tegmental radiations, the CTT is markedly thinned out, but still has a wide mediolateral extension. At the meso-diencephalic junction these disseminated



Fig. 4 Semidiagrammatic representation of the periventricular CA fibre system (rostral to the locus coeruleus), the medial fibre flow of the tegmental CA radiations (TR), and the CA fibres of the mamillary peduncle (MP). Composit drawing of somewhat different paramedian sagittal planes. For abbreviations, see Index.

CTT fibres bend ventrally through, and partly lateral to the medial lemniscus to run broadly into the zona incerta and Forel's H₂-field (Fig. 2). Here they run in a position and follow a course very similar to the myelinated fascicles of the incerto-tegmental and incerto-tectal tracts, as defined by e.g., Gurdjian [25] Bürgi and Bucher [11] and König and Klippel [31] Ventrally there is no clear delineation between these CTT fibres in the zona incerta and the outflow of fibres from the ventrolateral part of the tegmental radiations and the mesencephalic A 8 cell group as described in more detail below In the rostral mesencephalon and the caudal diencephalon, the lateral parts of the CTT pass along the crus cerebri and fibres leave the system ventrolaterally The most caudal ones run around the dorsal aspect of the crus cerebri, and the more rostral fibres run through the crus cerebri, towards the deep inner surface of the optic tract, into the supraoptic decussations. Further rostrally at the level of the subthalamic nucleus, remaining CTT fibres bend ventrally and laterally to run — partly intermingled with the internal capsule branch of the DTB (see above) — through the internal capsule towards the ansa lenticularis and the supraoptic decussations. These fibres might also reach the neostriatum and possibly the overlying cortex (cf Fig. 16).

C. THE DORSAL PERIVENTRICULAR SYSTEM

The GA method revealed a prominent CA fibre system extending in the periventricular and periaqueductal gray of the medulla oblongata, pons, mesencephalon and diencephalon It can be regarded as an adrenergic component of the dorsal longitudinal fasciculus, as described by Crosby and Woodburne [18] and Nauta and Haymaker [51] The most caudal fibres of the system were observed in a superficial position at the level of the nucleus of the solitary tract and the dorsal vagal nucleus. The fibres could be followed from this area, which is heavily supplied with fluorescent CA terminals and contains CA cell bodies belonging to the A 2 cell group as a rather sparse bundle running rostrally underneath the ependyma of the fourth ventricle. The direction of these fibres has not been established, it seems that they either could be axons ascending from the A 2 cell group or could represent descending fibres from the locus coeruleus cell group At the level of the locus coeruleus, the system increases considerably in width and in the number of fibres, in that the locus coeruleus and perhaps also other systems (e. g., the CA cell bodies distributed along the superior cerebellar peduncle group A 4) contribute ascending fibres to the system. The details of the arrangement of the DPS fibres in this region are obscured by the presence of a dense fluorescent terminal supply to the locus coeruleus and adjacent periventricular gray matter As noted above, it seems highly probable that these terminals arise from dorsally directed collaterals of the ascending CTT fibres. From the region dorsal and medial to the locus coeruleus, the DPS ascends rostrally underneath the ependyma occupying the lateral part of the periventricular gray Among the fibres are scattered CA-containing cell bodies that in

sagittal sections appear as a diffuse rostromedial extension of the locus coeruleus CA cell group. Supposedly these cells also project into the DPS.

Along its course underneath the fourth ventricle the DPS gives off fibres medially and in frontal sections, a continuous flow of fibres was observed crossing the midline just beneath the ependyma. It seems probable that these crossing fibres represent a broad pontine decussation of the DPS fibres. When these medial fibres reach the midline some of them turn sharply ventrally to run ventrorostrally along the raphe following a course parallel to the caudal surface of the decussation of the superior cerebellar peduncles ventrally they assume a rostral direction to ascend through the ventromedial tegmentum close to the interpeduncular nucleus. The course of this median pathway appears to be similar to that of the medial tegmentomamillary and the tegmentopeduncular tracts described by Ban and Zyo [3] in the rabbit and Briggs and Kaelber [10] in the cat. Somewhat more laterally (Fig. 4) these fibres were paralleled by another branch from the DPS running from the region of the locus coeruleus ventrorostrally through the lateral part of the medial longitudinal fasciculus. These fibres apparently give rise to a dense terminal network in the ventral tegmental nucleus, and then continue their ventrorostral course behind the decussation of the superior cerebellar peduncles. Like the median fibres they gradually bend rostrally to ascend — partly through the decussation — into the ventromedial tegmentum (Fig. 4). Interestingly this more lateral branch of the DPS follows a course that is conspicuously similar to that of the mamillary peduncle as described with classical neuroanatomical techniques [10, 12, 17, 61, illegible]. In addition to ascending fibres, it seems possible that the described CA pathways could comprise also descending fibres, originating in cells of the ventromedial tegmentum.

At the rostral end of the fourth ventricle, some of the DPS fibres sweep dorsally around the ventricular edge to run in a dorsocaudal direction into the posterior colliculus (Figs. 4 and 6). The laterally situated fibres in the DPS run rostrally along the mesencephalic root of the trigeminal nerve up to the decussation of the superior cerebellar peduncles. They run above and through the dorsal part of the superior cerebellar peduncle and turn ventrorostrally on its rostral side to join the so-called tegmental radiations (see below). The medial part of the DPS runs rostrally into a tangle of fluorescent fibres situated in the rostral part of the dorsal raphe nucleus and in the adjoining ventrolateral central gray This tangle appeared to comprise not only irregularly running nonterminal axons and scattered CA-containing cell bodies but also a terminal axonal network that seemed to arise from collateral branches of the ascending CTT fibres (see above). In the intact animal, it was not possible to trace the DPS fibres through this region, but there occur three major outflows from the fibre tangle that might at least partly represent the further continuation of the caudal part of the DPS. One outflow is dorsocaudally towards the posterior colliculus, located immediately rostral to the previously mentioned collicular projection from the DPS. A second outflow is ventrorostrally along the tegmental radiations. The third gives rise to a well-defined bundle running rostrally in the ventrolateral part

of the periaqueductal gray within the dorsal longitudinal fasciculus. This bundle named the dorsal periventricular bundle (DPB) is described in more detail in a parallel communication [37]. As illustrated in Fig. 4, the DPB — after having given off fibres to the anterior colliculus — gives rise to a diencephalic periventricular system that projects to medial and midline thalamic, epithalamic, pretectal and hypothalamic regions. As described below, the hypothalamic branch of the DPB most probably contributes to the so-called ventral periventricular system. The lesion experiments of Lindvall *et al.* [37] provide evidence that the DPB axons have their origin primarily in cell bodies (defined as the A 11 group) located among the DPB fibres and distributed along the periaqueductal and periventricular gray of the mesencephalon and the caudal thalamus (Fig. 5).

D. THE TEGMENTAL CATECHOLAMINE RADIATIONS

The term tegmental radiations (radiatio grisea tegmenti) was used by Welsschedel [60] and Kuypers [32] to describe the almost radially-oriented systems of unmyelinated or scarcely myelinated fibres in the mesencephalic tegmentum. These fibres are oriented in the frontal plane and when going from dorsal to ventral, they diverge strongly in the ventrolateral direction into a fanlike arrangement. When going from caudal to rostral, the frontal planes formed by the radiating fibres converge strongly ventrally into the region of the rostral, ventral mesencephalon (see ref. 3).

The tegmental radiations represent an architectonial principle in the mesencephalon that is of central importance for the understanding of the arrangement of the ascending CA fibre systems. Major portions of the medullary, pontine and mesencephalic fibre systems follow the tegmental radiations to assemble at the meso-diencephalic junction to form the ascending medial forebrain bundle and ventral periventricular CA systems. As pointed out above, all previously described ascending CA fibre systems — the DTB, the CTT and the DPS — and also mesencephalic CA cell groups, project through the tegmental radiations. Although CA fibres run ventrally and ventrolaterally along the tegmental radiations at all rostrocaudal levels of the mesencephalon (Fig. 1), the massive flow of fibres occurs in a rather narrow section, lying in between the decussation of the superior cerebellar peduncles and the red nucleus. This dense mesencephalic CA fibre arrangement will be referred to as the tegmental CA radiations, and it is illustrated in Fig. 7 and 8 in an oblique frontal section, falling in the plane of the radiating fibres, immediately rostral to the decussation of the superior

---

Fig. 5. Photomontages of the dorsal and ventral periventricular CA fibre systems in a horizontal and a paramedian sagittal section, respectively. *Above:* the dorsal periventricular bundle in the periaqueductal gray of the mesencephalon (AQ=aqueduct). Note the fluorescent cell bodies with a somewhat diffuse outline that project fibres rostrally (to the left) along the bundle. (X 190). *Below:* The ventral periventricular system ascending broadly along the lateral aspect of the periventricular hypothalamic nucleus, between the dorsomedial (dmh) and the paraventricular (pvh) nuclei. These latter nuclei are heavily supplied with fluorescent CA terminal networks. Cf. Fig. 4. (X 100).

AQ

dmh

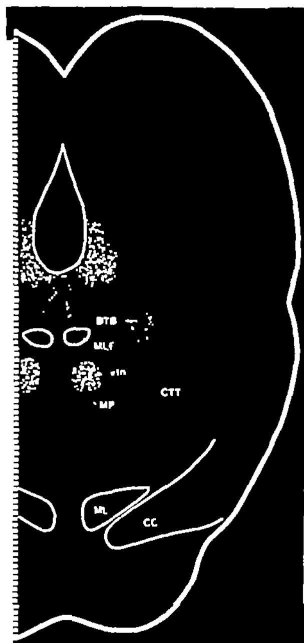


Fig. 6. Semidiagrammatic representation of the position of the ascending CA fibre systems in the dorsal tegmental bundle (DTB), the central tegmental tract (CTT), and the mamillary peduncle (MP) in a transverse section at the level of the ventral tegmental nucleus (vtn). For further abbreviations, see Index.

cerebellar peduncles. From the position of the radiating fibres, a median, a medial, and a lateral flow can be distinguished.

The median CA fibre flow is within and along the raphe. The fibres are very

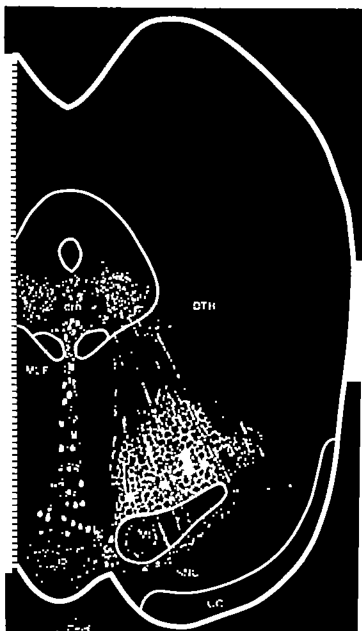
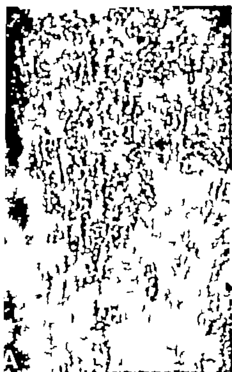


Fig. 7 Semidiagrammatic representation of the tegmental CA radiations in an oblique frontal plane, falling just rostral to the decussation of the superior cerebellar peduncles. Cf Figs. 1 2, and 4 For abbreviations, see Index.

delicate with fine spherical varicosities. They can be traced from the fibre tangle in the rostral part of the dorsal raphe nucleus in a rostroventral direction, between the two medial longitudinal fasciculi, down to the region dorsal to the interpeduncular nucleus, where most fibres are seen to turn sharply rostrally


A


B


C


D

Some of the fibres enter this nucleus from the dorsal side. Possibly some of the median fibres originate in the DPS but it seems highly probable that the most substantial contribution comes from the CA-containing cell bodies distributed along the raphe and located within the median fibre flow (the dorsal cells of the A 10 group). It is notable that an aggregation of these cells was located in the ventral portion of the dorsal raphe nucleus, a nucleus that is otherwise known to contain many indolamine-containing cell bodies [9, 19].

The medial flow of fibres runs in a ventrolateral (and somewhat rostral) direction from the fibre tangle in the dorsal raphe region in a position just lateral to the medial longitudinal fasciculus and medial to the DTB. Many of these fibres are assembled into strongly fluorescent fibre aggregates that split up into a characteristic network as they reach the ventral part of the tegmentum. Most probably part of these fibres terminate as strongly fluorescent varicose fibres in this region.

The lateral fibre flow is formed by the ventrolaterally turning DTB and CTT fibres, as described above. In sagittal sections, it was noted that part of the lateral fibres belong to a pathway running in the lateral part of the DPS from the locus coeruleus along the mesencephalic root of the trigeminal nerve turning ventrally in front of the superior cerebellar peduncle (cf. above). As illustrated schematically in Fig. 7 the lateral fibres run first ventrolaterally (and somewhat rostrally) into the region dorsal to the lateral part of the crus cerebri, where they might contribute to the adrenergic terminals present in this area. The laterocaudal group of mesencephalic CA cell bodies (the so-called A 8 group) is located in this ventrolateral part of the tegmental radiations, and the fibres of the lateral flow thus intermingle here with the outgoing fibres of the A 8 group. The further course of these axon systems was analysed in serial pseudo-sagittal sections, oriented about 45° to the sagittal plane. As already pointed out by Kuypers [22] this plane of section yields very valuable information on the further rostral course of the radiating fibres. It was thus revealed that the CA fibres of the lateral flow turn medially in the ventrolateral part of the tegmental radiations and intermingle with the axons of the A 8 cells. They run in medial and rostromedial directions to reach the ventral tegmental area of Tsai and the MFB on the dorsal and ventral side of the medial lemniscus. The more caudally situated fibres run straight medially dorsal to the medial lemniscus, and the more rostral fibres run rostromedially in between the medial lemniscus and the substantia nigra through the caudal zona incerta and the H₂-field of Forel, to join the MFB system in the caudal hypothalamus.

From the above description it is evident that there is a massive convergence

---

Fig. 8. Details of the CA fibre flows in the tegmental CA radiations photographed in the oblique frontal plane illustrated in Fig. 7. A. The median fibre flow in its position between the two medial longitudinal fasciculi. (X 260). B and C. The medial fibre flow ventromedial to the DTB. (X 225 and 240, respectively). D. Detail of the fibre arrangement in the ventrolateral part of the tegmental radiations (medial is to the left of the picture). (X 140).

of the CA fibres from the tegmental radiations at the meso-diencephalic junction in the region medial to the substantia nigra. Here together with fibres from the ventrally situated A 10 cells and probably also from some of the CA cells in the substantia nigra (group A 9), the converging fibres give rise to the ascending CA fibre systems within the MFB and the VPS (described below). Fibres also project to the mamillary complex, which in the GA material was revealed to have a much richer and more complex innervation than was previously observed with the Falck Hillarp technique. Three pathways were seen to contribute to this innervation. One is a direct rostral projection of very thin varicose fibres running superficially just above the interpeduncular fossa. Dorsal to the posterior mamillary nucleus, these fibres turn sharply ventral to give rise to a rich plexus of delicate varicose terminals in this nucleus. The second mamillary pathway appeared to arise from fibres situated more dorsally in the rostral projection from the median fibre flow. These delicate fine varicose fibres run rostrally in between the fibres of the ventral tegmental decussation (dorsal to the interpeduncular nucleus) past the fasciculus retroflexus on the medial side and down into the medial mamillary nucleus along the nonfluorescent myelinated fibres of the mamillotegmental tract. A third pathway to the mamillary complex is probably along the mamillary peduncle. As described above a branch from the DPS runs, from the region of the dorsal and ventral tegmental nuclei rostroventrally along the course of the mamillary peduncle. These fibres could not be traced through the dense fibre arrangements in the ventromedial tegmentum but at a more rostral level in its well-defined position in the mamillary region the peduncle was again observed to comprise many CA axons. They probably reach the mamillary nuclei from the lateral side and some of them may continue rostrally in the MFB system. The two latter pathways appear to be the main sources for the CA innervations of the medial and lateral mamillary nuclei. In addition the VPS was observed to give fibres to the medial nucleus as described below.

E. THE VENTRAL PERIVENTRICULAR SYSTEM

Caudally this CA fibre system was distinguished in the region immediately dorsolateral to the interpeduncular nucleus. The fibres run rostrally ventral to the supramamillary commissure and then medial to the mamillothalamic tract. In passing above the medial mamillary nucleus, fibres are seen leaving the bundle into the nucleus. Behind, through, and rostral to the mamillothalamic tract fibres from the medial part of the MFB leave this bundle partly as collaterals, rostromedially to join the VPS. The VPS thus increases in width and fibre number as it passes in a rostral and somewhat dorsal direction through the posterior hypothalamic area into the dense CA terminal area of the dorsomedial hypothalamic nucleus. Here the fibres meet those of the hypothalamic branch of the DPB which in all probability at least partly join the VPS, and form a broad ascending hypothalamic CA fibre system dispersed on the lateral aspect of the periventricular nucleus (Fig. 4). In paramedian sections,

the VPS could thus be seen to form a connexion between the CA term systems of the dorsomedial and the paraventricular hypothalamic r (Fig. 5). It seems possible that also the MFB system could contribute f c this part of the VPS via the branches of the MFB that project into the c medial and the paraventricular nuclei (Fig. 10 see below). Rostral to the p ventricular nucleus (Figs. 4 and 10), the VPS becomes dispersed mediolatera and some of the medial fibres turn dorsally to continue as periventricular th.err fibres. Most of the VPS fibres form a broad band that sweeps dorsally on the caudal and rostral sides of the anterior commissure, into the caudal septum and possibly also into the fornix towards the hippocampus. The most lateral part of the VPS runs rostrolaterally into the medial part of the CA terminal system in the interstitial nucleus of the stria terminalis. Here these fibres intermingle with fibres coming from the MFB (see below). Also these lateral VPS fibres might continue into the septum, running dorsally on the caudal and rostral surfaces of the anterior commissure. It is conceivable that other fibres could turn ventrolaterally to reach the MFB system. It has not yet been established, however whether the VPS projects further rostrally Nor has its terminal distributions within hypothalamus and septum been mapped. However it seems reasonable to suppose that the VPS contributes primarily to the CA innervations in the medial and periventricular hypothalamic areas and in the caudal septum. The existence of the periventricular CA fibre system, which has not been previously demonstrated, introduces new aspects on the organization of the hypothalamic and septal CA afferent systems. This will be pursued in more detail in further studies.

F THE MEDIAL FOREBRAIN BUNDLE SYSTEM AND THE NIGROSTRIATAL PATHWAY

Within the MFB a highly heterogeneous system of CA fibres ascends from the brain stem reticular formation towards diencephalic and telencephalic regions. As described in the previous paragraphs, both the DTB and the CTT have major routes of projection along the MFB and it is possible that also the DPS contributes fibres to this system. Another source of ascending CA fibres in the MFB is the mesencephalic CA cell groups, primarily the A 10 group, forming the so-called mesolimbic DA system [1, 44] Closely associated with the MFB system is also the nigrostriatal DA pathway which from previous studies [1, 14, 43, 47, 60, 64] is known to originate in the pars compacta of the substantia nigra (group A 9), and possibly also in cells located in the ventral tegmental area of Tsai (A 10) and in the more caudally situated A 8-group. Moreover there is recent evidence [8] that the substantia nigra DA cells project also along the MFB to innervate the anterior limbic cortex. The axons of the nigrostriatal and mesolimbic pathways were identified with an extremely delicate fluorescent axon type equipped with fairly regularly spaced, fine varicosities, which were characteristically arranged in fascicles. This characteristic fluorescence morphology of these presumed DA-containing axon systems made it possible to distinguish them from other CA axon systems running in the MFB. They could thus be traced from the

mesencephalic CA cell groups all the way up to their terminal areas, here the morphology of the axons changed as they ramified into terminal and paraterminal branches.

Caudally the CA fibre systems of the MFB and the nigrostriatal pathway assemble at the meso-diencephalic junction in the area dorsal to the mamillary peduncle and medial to the substantia nigra. The fibres assemble from the tegmental CA radiations, as described above via its strongly converging rostral outflow which carries fibres primarily from the medullary and pontine components of the CTT from th mesencephalic CA cell groups, and to a minor extent probably also from the DTB and the DP. It seems also possible that fibres running along the mamillary peduncle (see above) could continue rostrally in the MFB system in agreement with findings obtained with stains of anterograde degeneration [10, 45, 51]. Further rostrally the DTB joins the MFB in the middle hypothalamus, and at this level the ascending CA fibre system reaches its maximum thickness and abundance. The fluorescence morphology of the various CA axon systems allowed a rather safe distinction between the different ascending systems (cf. ref. 35), and their relative position in the bundle could therefore be observed. Interestingly the DTB and the CTT fibres retain in the MFB their relative positions in the pontine and mesencephalic tegmentum the DTB fibres running in the dorsomedial part of the MFB and the CTT fibres running ventrally and laterally to them. The mesencephalic (probably DA containing) MFB fibres occupy a lateral and somewhat dorsal position and they adjoin dorsally the more compact and fibre-rich nigrostriatal bundle which runs in a position immediately dorsolateral to the MFB along the medial edge of the crus cerebri and the internal capsule.

1 Presumed dopamine-containing systems

The nigrostriatal pathway (NSP) (Figs. _ and 10). The fibres from the CA cells in the pars compacta of the substantia nigra are in their initial course directed medially. Medial to the substantia nigra, they turn sharply rostrally and assemble in a well-defined bundle that ascends in the H₂-field of Forel immediately dorsolateral to the MFB system. The most dorsal fibres in the NSP leave the bundle first. At the level of the subthalamic nucleus, these dorsal fibres bend first sharply laterally above the crus cerebri, and then rostrally to turn into the internal capsule from the caudal side towards the caudal parts of the neostriatum. The somewhat more ventrally located fibres deviate less sharply and run in a

Fig. 9. Left. Photomontage of the medial forebrain bundle in a horizontal section similar to that illustrated in Fig. 10. The picture covers the bundle from the mamillary region (bottom) up to the level of the paraventricular hypothalamic nucleus (top). The branch projecting into the dorsomedial nucleus is seen to the right (X 85). *Right.* Sagittal section through the rostral hypothalamus showing DTB fibres in the dorsomedial part of the medial forebrain bundle. The fibres are seen to give off abundant collateral branches into the supraoptic decussations and towards the reticular thalamic nucleus and the stria medullaris (cf. Fig. 1). (X 160).

rostrolateral direction through the subthalamic region into the internal capsule. The centrally and ventrally located fibres continue rostrally along and partly within the dorsomedial edge of the internal capsule Along this course the more dorsally situated fibres in the bundle deviate in a rostro-latero-dorsal direction into the internal capsule towards the central parts of the nucleus caudatus-putamen The ventral portion of the NSP continues further rostrally up to the level of the globus pallidus. Here most of them fan out in rostrolateral and dorsal directions to run along the myelinated fascicles through the globus pallidus into the head of the nucleus caudatus-putamen The most ventral portion of the NSP however continues rostrally in a position just dorsal to the MFB up to the anterior commissure they pass ventrally to the commissure and medially and laterally to its anterior limb they run into the nucleus caudatus-putamen and the dorsal part of the interstitial nucleus of the stria terminalis. The ventral portion of the NSP also contributes fibres to the ansa lenticularis, as described below

The MFB systems. The presumed DA-containing fibre systems in the MFB run in a position immediately ventromedial to the NSP and they are not well demarcated from each other The several ascending DA systems appear rather as a single large bundle whose ventromedial portion runs within the MFB as this tract usually is defined in the classical neuroanatomical literature (see e. g., ref 33). The MFB fibres run in this position up to the level of the retrochiasmatic region where fibres leave the bundle laterally to run together with the fibres coming from the most ventral position of the NSP system *within the ansa lenticularis* — intermingled with presumed NA fibres — towards the amygdala, the caudal part of the ventral neostriatum, and the piriform cortex. It is notable that many of these DA fibres were observed to be collaterals from axons continuing rostrally The MFB system then separates into different components: One deviates slowly dorsally to run — dorsal to the MFB — in a rostral and somewhat dorsal direction *into the nucleus accumbens.* A second component takes a ventral position in the MFB In horizontal sections it was observed that these ventral fibres fan out in rostromedial and rostrolateral directions in the region just dorsal to the olfactory tubercle and from here turn ventrally *into the olfactory tubercle* Remaining fibres — constituting a major component of the system of presumed DA fibres in the MFB — continue rostrally within the MFB. Along its course this bundle gives off fibres that, together with presumed NA fibres, run in a dorsal and somewhat rostral direction *along the diagonal band into the septum* giving rise to a dense terminal system in the medial part of the lateral septal nucleus.

A minor portion of the bundle continues rostrally within the MFB up to the anterior part of the nucleus accumbens. Here the fibres run dorsally along the rostrolateral aspect of the accumbens and pass medially and laterally to the anterior limb of the anterior commissure. These fibres follow the external capsule, where they run in dorsal and lateral directions out on the surface of the nucleus caudatus-putamen and into the deep layers of the frontal cortex. CA fibres were seen to run in the external capsule also at more caudal levels; they seem to project both into the neostriatum and into the piriform cortex.

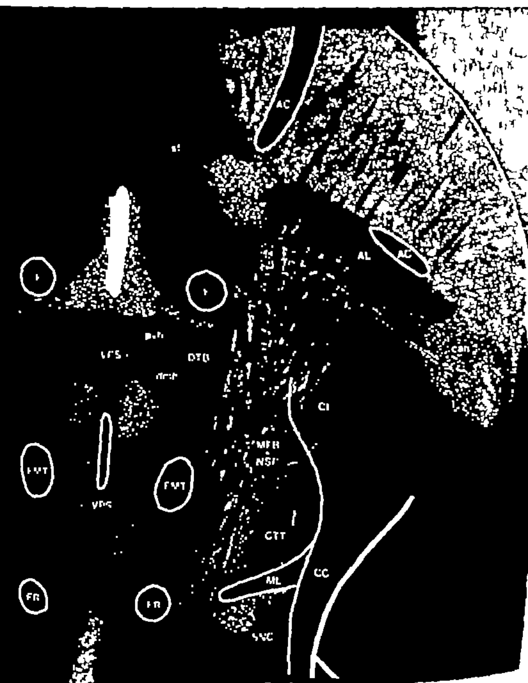


Fig. 10 Semidiagrammatic representation of a horizontal section through the dorsal part of the medial forebrain bundle system (MFB), the ventral part of the nigrostriatal pathway (NSP), and the ansa lenticularis (AL). Compare with the sagittal drawings in Figs. 1 and 2 and the frontal drawing in Fig. 12. Composite drawing of slightly different horizontal planes. For abbreviations, see Index.

The major portion of the bundle leaves the MFB at the level of the rostral septum in a dorso-medio-rostral direction to run in a position well corresponding to the septohypothalamic tract. The fibres sweep as a broad band along the medial and the medioventral aspects of the nucleus accumbens. The dense DA terminal system in this nucleus was not sharply demarcated from the bundle probably signifying a ramification from the bundle into the nucleus. In the region rostromedial to the nucleus accumbens the bundle separates into four main branches. a) The branch of greatest abundance runs dorsorostrally and laterally *into the deep layers of the frontal cortex* mingling with the above mentioned fibres passing rostrolaterally to the accumbens. These fibres give rise to an extensive DA-containing terminal system in the frontal cortex[6, 38] (Fig. 11). The branch to the frontal cortex passes along the rostral aspect of the external capsule and fibres are seen to run *into the external capsule* thus contributing to the above mentioned CA fibre system within the external capsule. b) A second branch of the bundle turns more sharply dorsally to run in a position caudal to the branch to the frontal cortex. It sweeps caudally above the corpus callosum *into the anterior limbic cortex* medial to the cingulum. As described in more detail by Lindvall *et al.* [38] and Björklund *et al.* [6] these fibres give rise to an abundant dopaminergic terminal system in the anterior limbic cortex. c) A third branch runs in a dorsocaudal direction *into the septum* contributing to the innervation of this area. This branch comprised both collaterals and fibres deviating from the bundle. d) Fibres in a ventral position in the bundle turn first dorsally for a short distance and then rostrally to run *within the medial olfactory tract*. Presumably these fibres give rise to terminals in the olfactory nuclei.

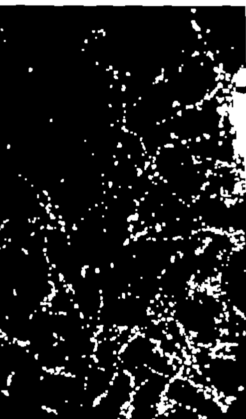
Presumed noradrenaline-containing systems

Probably reflecting their highly diversified origins, the morphology of the presumed NA axons in the MFB is quite heterogeneous. The locus type of preterminal axon reaching the MFB via the DTB and possibly also via the CTT (see above) has a characteristic appearance and can thus be identified in the fluorescence microscope. The non-locus types, on the other hand, consist of beaded fibres of much varying appearance and widely varying calibres. In the following description the different morphological types of non-locus fibres therefore will not be distinguished (cf. Fig. 9).

The preterminal axons of *the locus type* run predominantly in the dorsomedial part of the MFB. In the rostral hypothalamus, the axons give off abundant

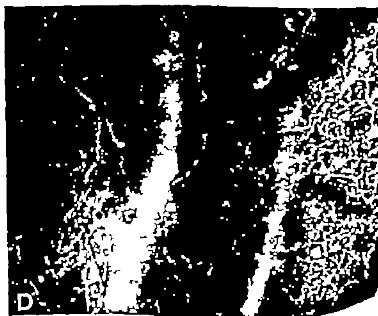
---

Fig. 11. Details of the two principal ... on types innervating the neocortical areas. A. Varicose terminals of the locus coeruleus, presumed noradrenaline-containing type in the molecular layer of the sensory-motor cortex. (X 400). B. Presumed dopamine-containing axon terminals in the deep layers of the frontal cortex. (X 260). C. Nonterminal axons of the locus coeruleus type in the rostral part of the septum running towards the cingulum. (X 350). D. Nonterminal presumed dopamine-containing axons running on the rostral side of the neostriatum (whose dense fluorescent terminal network is seen to the right in the picture) towards the frontal cortex. (X 350).




B




D

collaterals ventrally and dorsally. Of the dorsally directed collaterals the caudal ones run into the reticular thalamic nucleus, and the more rostral collaterals form a bundle which runs on the dorsal surface of and partly also within *the stria medullaris*. This bundle contributes to the adrenergic innervation of the anterior thalamic nuclei. The ventrally directed collaterals mix with collaterals of the non locus axons to run into *the supraoptic decussations* (see below).

Somewhat further rostrally locus and non-locus fibres leave the MFB dorsally into the ventral part of the *interstitial nucleus of the stria terminalis*, which is heavily supplied with fluorescent CA-containing varicose fibres. They represent to a large extent at least the ramifications of the non locus fibres (refs. 55, 64, unpublished observations), whereas the locus fibres appear to pass through the nucleus into the *stria terminalis*, *the fornix* and *the caudal septum*. Locus fibres were also seen to run medially across the midline along the ventral surface of the anterior commissure. Within the stria terminalis, the axons were followed all the way to the amygdala. Dorsally, when passing close to the head of the nucleus caudatus-putamen at the level of the fornix commissure, fluorescent fibres were seen to leave the stria terminalis rostrally through the internal capsule into the caudate nucleus. The locus fibres running in the fornix were traced into the hippocampus.

Remaining locus fibres continue rostrally in the MFB up to the level of the rostral septum. Here, some fibres continue rostrally in a position ventrolateral to the intermediate olfactory tract into the lateral part of the anterior olfactory nucleus. The major portion, however, turns dorso-medio-rostrally to run close to the previously described fibres of the DA-containing type along the septohypothalamic tract (Fig. 11 C). Just ventral to the genu of the corpus callosum, the bundle of locus fibres divides into a caudal and a rostral branch. The caudal branch was traced underneath the corpus callosum in *the fornix superior* all the way back to the hippocampus. The rostral branch runs caudally above the corpus callosum within *the cingulum*. This branch gives off fibres along its course to large areas of the neocortex, and caudally it sweeps around the splenium of the corpus callosum to enter the hippocampus from the caudal side.

The preterminal fibres of *the non-locus types* occupy a position in the MFB ventral and lateral to the locus fibres. Along their course through the hypothalamus and the preoptic region, they give off abundant collaterals that contribute importantly to the adrenergic innervations of these regions.

a) From the most ventral part of the MFB, a loosely arranged system of collaterals leaves the bundle in a ventromedial direction along the ventral brain surface towards *the mediobasal hypothalamic area*. Part of these fibres most probably enter the median eminence, constituting *the so-called reticulo-infundibular NA pathway* as described by Björklund *et al.* 8.

b) Abundant collaterals of non-locus fibres leave the MFB system medially at two levels in the hypothalamus (Fig. 10). First, broadly into *the dorsomedial nucleus* and second, as a well-defined branch that projects medially and somewhat rostrally from the dorsal part of the MFB into *the paraventricular hypo-*

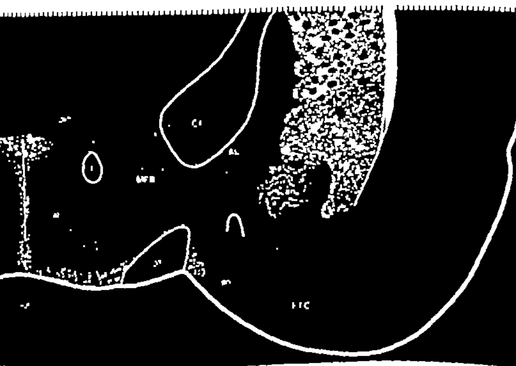


Fig. 12. Semidiagrammatic representation of the CA fibre systems running in the supraoptic decussations (SOD) and the area lenticularis (AL) as observed in a frontal section through the retrochiasmatic region. Cf Fig. 10. Composite drawing of slightly different frontal planes. For abbreviations, see Index.

thalamic nuclei. They most probably contribute importantly to the dense adrenergic innervations of these nuclei.

c) At the level of the retrochiasmatic region, abundant fibres leave the MFB ventromedially to pass on both sides of the fornix into *the supraoptic decussations* (SOD). The highly interesting arrangement of these projections is illustrated semidiagrammatically in Figs. 12 and 13. The fibres decussate — intermingled with the decussating locus fibres — within the SOD and along their course they give off collaterals dorsomedially into the anterior hypothalamic area, dorsally into the anterior periventricular nucleus (Fig. 12), rostrally as a broad band of fibres past the optic chiasm on its dorsal aspect (Fig. 13), and probably to a minor extent also caudally into the retrochiasmatic region. In animals subjected to a total unilateral destruction of the ascending pontine and medullary CA fibre systems (unpublished observations) it was revealed that these collaterals were given off from the decussating fibres on both sides of the midline. The most medial of the rostrally directed fibres run medial to the suprachiasmatic nucleus into the periventricular preoptic region. The more lateral fibres fan out in rostral and rostrolateral directions, giving rise to axonal ramifications in the ventral part of the preoptic area and in the supraoptic nuclei. Some fibres sweep just rostral to the supraoptic nucleus out laterally into the piriform cortex. The

decussating fibres continue laterally and caudally within the SOD along the dorsomedial surface of the optic tract. Part of the fibres deviate laterally into the ansa lenticularis (see below) and others turn laterally and ventrally into the posterior part of the supraoptic nucleus (Fig. 17). Fibres, primarily of the locus type were traced along the optic tract all the way back to the region of the metathalamus. Although their direction was not established it seems probable that part of them represent caudally running axons from the SOD contributing to the innervation of the geniculate bodies. In addition to the inflow from the MFB, CTT and DTB fibres reach the SOD also from the zona incerta through the internal capsule and caudally from the lateral midbrain tegmentum as pointed out above but their courses and terminations were not established.

d) A major projection of the MFB system is along the *ansa lenticularis*. As illustrated in Figs. 10 and 12, part of these fibres are aggregated into a rather dense bundle which leaves the MFB in a lateral direction at the level of the retrochiasmatic region. The bundle comprises fibres of both the DA and the non locus NA types many of them being identified as collaterals of the fibres continuing rostrally within the MFB. The bundle ran laterally within the ansa lenticularis — receiving further fibres coming from the SOD and also from the zona incerta through the internal capsule (cf. above) — into the caudal part of the ventral neostriatum and the amygdala (particularly the central amygdaloid nucleus).

Further rostrally in the suprachiasmatic and preoptic regions, there is a lateral flow of loosely arranged disseminated fibres, primarily coming from the ventral part of the MFB and from the rostrally projecting collaterals of the SOD (cf. above). The course and position of this disseminated fibre path correspond well to the so-called amygdalofugal pathway as described e. g. by Valverde [63] and Nauta and Haymaker [81]. The CA fibres in this pathway run laterally and caudally to project broadly into the amygdaloid piriform region. The most medially located fibres could be seen to enter the subiculum of the hippocampus, signifying another route of projection of the CA afferents to the hippocampus.

e) Somewhat further rostrally non locus fibres leave the MFB dorsally and slightly medially into *the ventral part of the interstitial nucleus of the stria terminalis* together with fibres of the locus type as mentioned above. The non locus fibres ramify abundantly within the nucleus, but it seems possible that they could continue past the anterior commissure into the septum. In between the two interstitial nuclei fibres cross the midline along the ventral and caudal surfaces of the anterior commissure partly intermingled with the above-mentioned decussating locus fibres. Decussating fibres, possibly belonging to this system were also detected within the anterior commissure.

f) In the preoptic region fibres leave the MFB in medial and ventromedial directions into *the medial preoptic area* and along *the diagonal band towards the septal nuclei* (Fig. 13).

An extension of non-locus fibres beyond the preoptic and septal regions uncertain from the present observations. It seems possible however that

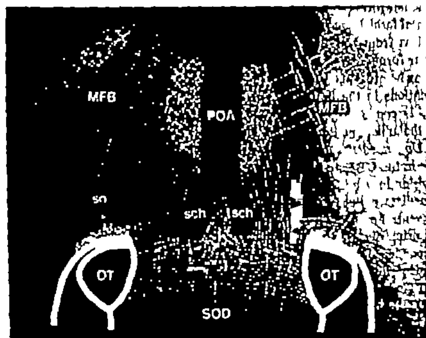


Fig. 13 Semidiagrammatic representation of the CA fibres in the supraoptic decussations (SOD) and their rostrally and caudally projecting collateral branches as seen in a horizontal section falling immediately dorsal to the optic chiasm. Fibres with abundant collaterals running in the most ventral part of the MFB system are also seen in the preoptic region. For abbreviations, see Index.

fibres could reach, e. g. the olfactory bulb but this can only be verified through further experimental work.

DISCUSSION

The ascending CA fibre systems from the mesencephalic, pontine, and medullary reticular formation are principally associated with four major and important conduction pathways. *the periventricular fibre system the central tegmental tract the medial forebrain bundle system*, and *the nigrostriatal pathway* To a very large extent the adrenergic pathways follow — along or reciprocal to — well established non-adrenergic fibre tracts. Thus a number of fibre systems, well known from the classical neuroanatomy carry also adrenergic axons, for instance the fasciculus retroflexus, the mammillo-tegmental and mammillo-thalamic tracts, the stria medullaris, the stria terminalis, the fornix, the mammillary peduncle, the ansa lenticularis, and the supraoptic decussations. Therefore it seems both feasible and desirable to adapt as far as possible the nomenclature and description of the CA systems to the prevailing neuroanatomical terminology

The central tegmental tract (CTT) offers some special problems in this respect. In the neuroanatomical literature, the CTT is usually identified with a highly heterogeneous and partly loosely arranged system of longitudinally running fibres in

the reticular formation. The tract, whose fibres follow the characteristic myelinated tegmental fascicles, comprises both ascending systems from the reticular formation as well as from the spinal cord [45, 82, 88] and several descending systems to the reticular formation [12, 18, 41, 64] The ascending adrenergic fibres in the CTT have a similar heterogeneous and partly loose arrangement. On the basis of origins and *terminations in the brain, the CA fibre system, designated CTT in the present* paper is composed of many different axonal pathways (e. g. ponto-cortical, ponto-thalamic, ponto-hypothalamic ponto-tectal, bulbo-hypothalamic fibres, etc.). Ungerstedt [84] and Olson and Fuxe [65] distinguished a dorsal and a ventral bundle in the CTT and Maeda and Shimizu [42] and Maeda et al. [41] made a further subdivision into a dorsal pathway (originating in the principal locus coeruleus), an intermediate pathway (originating in the disseminated pontine CA cell bodies), and a ventral pathway (originating in the medullary cell groups). This subdivision, made primarily on the basis of the connexions of the fibres, did not have a correspondence in distinguishable separate bundles in the present material. In agreement with Ungerstedt's [84] description, the fibres in the dorsomedial part of the CTT system were characteristically aggregated into a so-called dorsal bundle (the DTB) but this bundle did not carry all CTT fibres originating in the principal locus coeruleus. Remaining parts of the CTT have a more complex organization involving a considerable overlap between the pathways originating in the disseminated pontine CA cells, and in the medullary cell groups. These pathways should therefore be regarded as mutually overlapping components of the CTT system, as illustrated schematically in Fig. 16.

Also the MFB is highly heterogeneous with respect to CA fibres. This conduction pathway is a route of projection of fibres from practically all ascending NA and DA fibre systems. However as revealed in the present study the MFB is by no means the sole route of projection for the CA fibre systems to the di- and telencephalon. Thus, both the periventricular system, the CTT and the DTB — which partly contribute fibres to the MFB system — have important projection pathways also outside this system. It is also clear that the composition of the MFB, with respect to its CA fibres, differs considerably at different levels: in the posterior hypothalamus, in the anterior hypothalamus, or in the preoptic region.

THE PERIVENTRICULAR SYSTEM

This prominent CA fibre system — illustrated diagrammatically in Fig. 14 — is distributed along the periventricular and periaqueductal gray from the medulla oblongata up to the rostral diencephalon and the septal region. In the present description, a distinction is made between a dorsal system of fibres — extending along the dorsal longitudinal fasciculus, taken in its widest sense — and a ventral system extending along the periventricular region of the hypothalamus. The rostral, mesencephalic and diencephalic, part of the dorsal system forms a well-defined bundle the DPB that has previously been observed by Richardson and Jacobowitz [70] in rats lesioned with 6-hydroxydopa. The DPB projects to medial

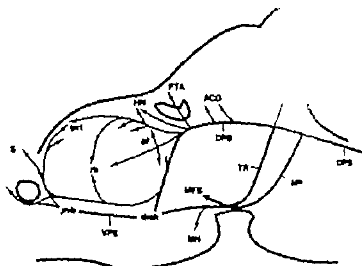


Fig. 14 Schematic representation of routes of projection of the periventricular and paramedian fibre systems to the tectum, diencephalon, and septum. Compare with Fig. 4 and text. For abbreviations, see Index.

and midline thalamic, epithalamic, pretectal, and hypothalamic regions along routes that fall essentially within the periventricular fibre system of Krieg [39]. For a more detailed account of the organization of the DPB system, the reader is referred to the paper by Lindvall *et al.* [37]

In its arrangement the dorsal periventricular system (DPS) is quite different from the other CA systems studied, many of its cell bodies of origin being distributed diffusely along its extent through pons, mesencephalon, and the posterior thalamus. This signifies that axons are contributed to the bundle all along its extent in these regions. In addition the locus coeruleus was seen to project significantly rostrally along the DPS, and it seems possible that also the A 4 and A 2 CA cell groups and the CA cell bodies present in the area postrema [38] could contribute too. Moreover it seems possible that the DPS could contain also descending CA projections, particulary in the caudal parts of the system, but so far there are no observations to substantiate this assumption. The arrangement of the CA cell bodies in the DPS suggests that this adrenergic system — similar to what has been described for the non-adrenergic neuron systems in the dorsal longitudinal fasciculus [18, 43] — may be composed of relatively short fibre systems that originate at different levels of the DPS and run for some distance along the bundle to terminate locally in the central gray or to leave the bundle at successively higher levels. With this arrangement it would seem improbable that any of the CA fibres would ascend in the DPS along its entire extent. In fact, Lindvall *et al.* [37] did not observe any significant removal of DPS fibres in the mesencephalic and thalamic parts of the system (i. e. the DPB) after a complete lesion of the DPS at the level of the dorsal raphe nucleus.

It is clear that the DPS carries prominent ascending CA systems projecting to tectal, pretectal, thalamic, epithalamic hypothalamic and possibly also septal regions. In addition the DPS together with the dorsal part of the mesencephalic A 10 cell group gives rise to a paramedian fibre system running rostroventrally along and lateral to the midbrain raphe probably contributing to the adrenergic innervation of the mamillary nuclei, to the ascending MFB system, and possibly also to the VPS. In part these paramedian mesencephalic CA fibres seem to constitute a component of the mamillary peduncle (cf refs. 10 13 17 51 and 52) which has been shown with classical neuroanatomical techniques to carry ascending fibres from the region of the dorsal and ventral tegmental nuclei to the mamillary nuclei [2, 10, 17, 45, 52]. With stains for anterograde degeneration Nauta and Kuypers [52] Morest [45] and Briggs and Kaelber [10] have demonstrated a projection of the mamillary peduncle also further rostrally along the MFB to the lateral hypothalamic preoptic, and septal areas.

Ascending projections within the dorsal longitudinal fasciculus from the midbrain periaqueductal gray have been demonstrated with the Nauta and Fink Heimer methods by several authors [16, 24, 27, 45, 51, 52, 61]. The routes of projection in the diencephalon of the ascending fibres, as revealed with these staining techniques, are notably similar to those of the adrenergic DPB described in the present paper (cf ref 37). It is thus evident that the adrenergic DPB system is a major component of the ascending dorsal longitudinal fasciculus. Constituting major connexions from Nauta's [50] "limbic midbrain area" the ascending periventricular fibre system and the mamillary peduncle have been presumed to play important roles in hypothalamic endocrine functions [50, 51, 61]. The periventricular system has also been thought to be involved in such central functions as rage reactions, motor activity and food intake (for review of literature see Hamilton [26]). Against this background the discovery of the prominent periventricular and paramedian CA fibre systems should be of great interest in the studies of the role of CAs in the functions of the reticular formation.

THE CENTRAL TEGMENTAL TRACT

As indicated above this term is used to describe the heterogeneous and partly loosely arranged longitudinal CA fibre system that courses with Forel's tegmental fascicles through the brain stem reticular formation. This fibre system comprises both ascending and descending pathways, and it has rich local projections within the lower brain stem and to the cerebellum that are not dealt with in the present description. On the basis of the fluorescence morphology of the CA axons, *projections from the principal locus coeruleus* and *projections from the disseminated pontine and medullary CA cell groups* could be distinguished.

Ascending projections from the locus coeruleus were observed along three routes: a) along the DTB b) as disseminated fibres along the medial part of the CTT and c) within the periventricular gray of the fourth ventricle, along the DPS. Some of the ascending locus fibres may cross the midline in the prominent decussation observed ventral to the nuclei. There was also a prominent *descending*

pathway running dorsomedially in the pontine and medullary portion of the CTT and from previous studies in other laboratories the locus coeruleus is known to project along the superior cerebellar peduncle to the cerebellum [16, 54, 58, 61] The DTB is the most abundant of the three ascending pathways, and its CA-containing axons seemed to be exclusively of the locus coeruleus type. In studies with the Falck Hillarp technique in conjunction with lesions, the DTB has previously been identified as an important projection route of the locus neurons by Ungerstedt [64] Olson and Fuxe [54, 55] Maeda and Shimizu [42] and Maeda *et al.* [41] The latter authors observed in the cat also the projection from the rostral locus coeruleus along the DPS. Ascending reticular projections from the locus coeruleus region of the pons have also been observed with the Marchi stain for degenerating myelin by Russell [56] and Kuru and Yamamoto [31] in the cat. Interestingly these fibres, which can be presumed to be non-adrenergic in nature, run partly in a position that seems to be identical with that of the adrenergic DTB Moreover from the studies of Bürgi and Bucher [12, 13] in the cat, it seems that also descending myelinated pathways (the so-called thalamo-pretecto-tegmental fibres) run within this bundle. Thus, in its extension through pons and mesencephalon, the DTB appears to be a fairly well-defined, fibre-rich fascicle within the CTT carrying both ascending and descending, myelinated and non-myelinated fibre systems one of which is the ascending adrenergic locus projection.

In the GA-treated specimens, the dorsal tegmental CA bundle was traced through the zona incerta into the dorsomedial part of the MFB in the middle hypothalamus. Here, it possibly joins locus fibres that have ascended within the CTT and through the tegmental radiations to reach the MFB at more caudal levels. Within the MFB the locus fibres then distribute to many diencephalic and telencephalic areas, as illustrated diagrammatically in Fig. 15 In agreement with previous fluorescence histochemical and autoradiographic studies [27, 42, 57, 58, 64] the major areas of termination of the system in the di- and telencephalon were found to be thalamus, neocortex, and hippocampus. The ascending locus axons were seen to project also to the tectum, pretectum, metathalamus, amygdaloid-piriform region, and probably to the septum and the olfactory bulb. These observations made in the intact animal have partly been confirmed in animals subjected to a bilateral destruction of the locus coeruleus (ref 37 unpublished observations). At variance with the observations of Loizou [39] and Ungerstedt [64] however no significant termination of locus fibres could be established in the hypothalamus.

The rich collateralization of the locus coeruleus axons was directly observed in the GA-treated material, confirming the previous presumptions made on indirect evidence by Olson and Fuxe [54] and Ungerstedt [64] Thus, for example the DTB axons were seen to give off collateral branches towards the tegmental, tectal, and metathalamic regions, and further rostrally into the supraoptic decussations (towards metathalamic and pretectal areas) and the reticular and anterior thalamic nuclei (along the stria medullaris). Remaining axons then swept caudally in the cingulum to terminate in cortical and hippocampal areas. This branching

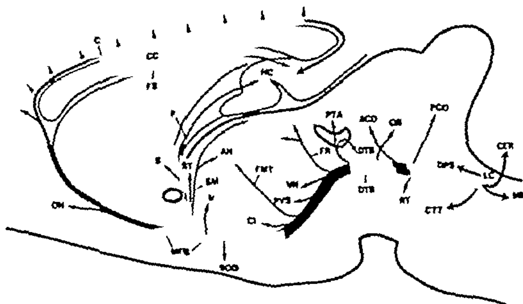


Fig. 15 Schematic representation of the course and primary branchings of the dorsal tegmental bundle originating in the CA cell bodies of the principal locus coeruleus. Compare with Fig. 3 and text. For abbreviations, see *Index*.

pattern suggests that one and the same locus neuron could influence — through collateral branches — several different regions in the brain. It is perhaps of particular interest that the widespread projections of locus coeruleus axons to both the specific thalamic nuclei and the neocortex provide a morphological basis for a representation of the activity of these neurons on both the cortical and the thalamic levels. In this context it should be noted that the rich collateralization is not unique for the locus coeruleus CA neurons, but a prominent feature also of the ascending projections from other pontine and medullary CA cell groups.

The ascending projections from the disseminated pontine and *groups* — together with fibres from the locus coeruleus — form arranged fibre system in the CTT It corresponds to ... d ventra as defined by Ungerstedt [41] and Olson and ... icated ment of the ascending bundles within this present material — is illustrated diagram ... of origin of this system are situated in the A 1 A 5 cell groups of Dahlström and Fuxe [15] ... pro buted to the CTT system also from th extension of the coeruleus-subcoerul ... sy cerebellar peduncle into the roof of Such an arrangement would agree w retrograde changes in the A 4 cells cell group. In agreement with the

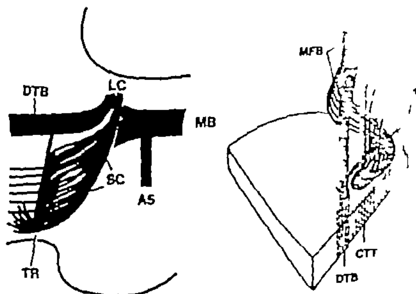


Fig. 16. The course of the CA fibre systems of the central tegmental tract through the so-called tegmental CA radiations in the mesencephalon as represented schematically in a lateral view (left) and in a dorsal view (right). Compare with Figs. 2 and 7 and text.

medullary portion of this system was observed to run dorsomedially in the CTT just ventrolateral to the DTB.

The CTT contributes importantly to the formation of the so-called tegmental CA radiations, from which the CA systems assemble at the meso-diencephalic junction to form part of the ascending MFB system (Fig. 16). The complex fibre arrangement in this region of the mesencephalic tegmentum, situated just rostral to the decussation of the superior cerebellar peduncles, consists partly of radiating preterminal axon bundles — following the characteristic fibre architecture of this part of the tegmentum [22] — and partly of densely arranged varicose terminal axonal ramifications. The dense CA terminal arrangement of this region has previously been described as the "nebula-like" formation by Maeda *et al.* [41] in the cat brain. The principal courses of different components of the CTT in this region are schematically illustrated in Fig. 16. Rostral to the tegmental radiations there are at least three different components of the CTT taking different projection routes. A ventral component, partly intermingled with the axons from the mesencephalic A 8 cell group, runs just dorsal to the substantia nigra to join the MFB system in the region medial to the substantia nigra, and along the MFB the CTT fibres reach hypothalamic, thalamic, preoptic, septal, amygdaloid, and piriform regions, a lateral component passes along the optic tract within the SOD a rostral component runs through the zona incerta and Forel's H₂-field along a course corresponding to the so-called incerto-tegmental and incerto-tectal tracts (cf refs. 13 25 33). This latter component runs broadly into the internal capsule towards the amygdaloid-piriform region, the neostriatum, and possibly also into the neocortex. In addition fibres were

observed to run into the SOD The distributional pattern of the MFB component of the non locus CTT fibres conforms to that ascribed to the so-called ventral CA bundle by Ungerstedt [64] and Olson and Fuxe [65] and to the so-called intermediate and ventral bundle by Meada *et al* [41, 42] In addition Maeda *et al* [41] concluded from lesion experiments in the cat that the so-called intermediate pathway of the CTT would give terminals also to the occipital cortex and Richardson and Jacobowitz [59] and Sachs *et al* [57] in rats and mice treated with 6-hydroxydopa, observed presumed NA axons running through the internal capsule and the neostriatum into the overlying neocortex. In line with these observations, both the DTB and the zona incerta component of the CTT gave off fibres that ran broadly into the internal capsule partly intermingled with DA fibres of the nigrostriatal pathway These axons could partly be traced into the neostriatum (probably giving rise to the coarse varicose type of fluorescent terminal found scattered in this area), but in the GA treated material, the number of fibres that ran through the external capsule into the neocortex having a fluorescence morphology of NA-containing axons of the locus or non-locus types, were sparse although such fibres were observed. Thus, in the rat, the GA method does not provide support for a *substantial* neocortical NA innervation via the internal capsule and the neostriatum.

An important decussation of fibres of both the locus and the non-locus types was revealed within *the supraoptic decussations* (SOD): this agrees with the earlier findings of Maeda *et al* [41] in the cat. The CA fibres ran into the SOD along three different routes: a) Caudally in the rostral mesencephalon and the caudal diencephalon, fibres in the lateral part of the CTT curved around or through the crus cerebri towards the medial surface of the optic tract, i.e. along a course similar to that of the (partly myelinated) fibres of the ventral SOD [11, 45] These lateral CTT fibres could therefore be regarded as an adrenergic component of this decussation. b) In the rostral hypothalamus, collateral branches from fibres of the locus and non-locus types left the MFB system ventromedially into the ventral and dorsal SOD These fibres gave off abundant collaterals innervating large areas of the anterior and preoptic hypothalamic regions, including the supraoptic nuclei (see Figs. 12 and 13). The decussating fibres continue along the dorsomedial surface of the optic tract to project along the ansa lenticularis towards the amygdaloid-piriform region others might continue further caudally along the optic tract towards the metathalamic and pretectal regions. c) Fibres leave the DTB and the CTT in the subthalamic region and run ventrally and laterally through the internal capsule into the SOD Within the SOD the CA fibres from the various sources intermingled, thus it was not possible to determine in the normal intact animal, the exact course and termination of the different components of the decussating CA fibres. This obviously requires further experimental studies.

With the Marchi and Nauta stains of anterograde degeneration Bucher and Bürgi [11] Zyo *et al* [69] and Minderhoud [45] have demonstrated fibres in the dorsal SOD that originate in the pontine reticular formation and ascend along the medial longitudinal fasciculus and through the zona incerta to decussate

the retrochiasmatic region. Zyo *et al* [49] also traced fibres, after lesions in the midbrain and pontine tegmentum, along the ventrolateral part of the MFB into the ventral SOD These systems, which are at least partly myelinated are in all probability non-adrenergic. The course of the decussating adrenergic DTB and CTT fibres appears to be parallel to the decussating non-adrenergic systems and the adrenergic and non-adrenergic systems seem to intermingle in the rostral hypothalamus. From the observations in the quoted studies [11, 44, 65] it seems in fact possible that the adrenergic and non-adrenergic components of the ventral and dorsal SOD to some extent have similar projections.

DOPAMINE-CONTAINING SYSTEMS

The mesencephalic CA cell groups — groups A 8 A 9 and A 10 of Dahlström and Fuxe [18] — are known to give rise to major ascending projections to the neostriatum and the limbic forebrain, and most of these neurons, at least are known to be DA-containing [1, 3, 18, 64]. Of these neuron systems, the nigrostriatal pathway — arising in cell bodies located in the substantia nigra (mainly within the pars compacta) and presumably also in the ventral tegmental area and in the A 8 cell group — has been established with a large variety of techniques, including fluorescence histochemistry biochemistry stains of terminal degeneration, and electron microscopy (for review of the literature see Moore *et al* [47] and Carpenter and Peter [14]). In addition, studies with the Falck Hillarp technique in conjunction with lesions have provided evidence for a projection of the mesencephalic DA cell groups (primarily the A 10 group) along the MFB to the nucleus accumbens, the olfactory tubercle and the interstitial nucleus of the stria terminalis [1, 64]. Ungerstedt [64] named these latter projections the mesolimbic DA system.

The characteristic fluorescence morphology of the presumed DA-containing axons in the GA-treated material made possible their distinction already in the normal untreated animal. In agreement with previous observations the ascending DA fibres of the nigrostriatal and the mesolimbic pathways could thus be traced from the mesencephalic DA cell groups all the way up to the neostriatum and to the accumbens, olfactory tubercle and the interstitial nucleus of the stria terminalis respectively. Also previously unknown presumed DA containing pathways were traced from the region of the mesencephalic cell groups to the septum, the frontal and anterior limbic cortices, the olfactory bulb and — via the ansa lenticularis — to the amygdala and the piriform cortex, the GA method thus disclosing far more wide-spread telencephalic projections of the mesencephalic DA cell groups than were previously known. The organisation of the nigrostriatal mesolimbic and mesocortical DA systems is schematically represented in Figs. 17 and 18.

The fibres assemble ventromedially at the meso-diencephalic junction and ascend as rather well-defined bundle, situated within and immediately lateral to the MFB. The different components within this system were found to have an interesting topographic arrangement. The fibres going to the

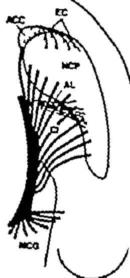


Fig. 17. Schematic representation of the presumed dopaminergic mesolimbic and mesocortical systems in a sagittal projection. For abbreviations, see Index.

Fig. 18. Schematic representation in a horizontal plane of the presumed dopaminergic projections from the mesencephalic CA cell groups (MCG) to the nucleus caudatus-putamen (NCP). Compare with Figs. 2, 10, and 12, and text. For abbreviations, see Index.

occupy the most dorsolateral part, then come the fibres to the interstitial nucleus of the stria terminalis, the fibres to the accumbens, the fibres to septum and the cortical areas, and most ventrally the fibres to the olfactory tubercle. Also within the nigrostriatal pathway there appeared to be at least a crude topographic arrangement, in that the fibres going to the caudal regions lie more dorsally and laterally and those going to the rostral and ventral regions lie more ventrally This probably reflects the topographical relation demonstrated between the cells of the pars compacta of the substantia nigra and the neostriatum [3, 14, 44]

Recently Thierry et al. [82, 83] *presented biochemical evidence of a dopaminergic* innervation of the neocortex, this has subsequently been supported by fluorescence histochemical and pharmacological observations [28, 31] In a parallel study using the GA method combined with stereotaxic lesions and CA determinations,

Lindvall *et al.*[34] and Björklund *et al*[6] demonstrated abundant DA-containing terminal systems in the frontal and the anterior limbic cortices which are the terminal projections of the cortical component of the ascending mesencephalic DA system described in the present paper According to the lesion experiments, the fibres innervating the frontal cortex originate in the A 10 cell group and those innervating the anterior limbic cortex in the lateral part of the substantia nigra (i. e. the A 9 cell group). In contrast to the previously established noradrenergic neocortical innervation originating in the locus coeruleus[22, 64] these mesocortical dopaminergic pathways seem to have restricted areas of projection in the neocortex and their terminal density is considerably higher Obviously the discovery of the extensive telencephalic projections of the mesencephalic dopaminergic neurons introduces entirely new aspects on the role of DA in the brain, these pathways providing alternative routes for brain stem influences on the neocortical functions.

GENERAL CONSIDERATIONS

The controversy that has existed regarding the projections of the monoamine neuron systems in the brain stem (see Moore[46]) resulted from the failure to trace with neuroanatomical techniques many of the telencephalic pathways that have been inferred from fluorescence histochemical and biochemical data. With respect to the nigrostriatal pathway the controversy was settled through the application of the more sensitive Fink Heimer and Wiltanen techniques for degenerating terminals, which allowed the direct tracing of this pathway[11, 43, 44] Subsequently the nigrostriatal DA pathway has also been traced with the Falck Hillarp method in newborn mice[34] and in human fetuses[53] However also the more sensitive neuroanatomical staining techniques have failed to demonstrate significant ascending projections from the brain stem reticular formation to the telencephalon, most notably the neocortex and the limbic forebrain structures[18, 40, 51, 52, 61] although there is substantial evidence of such projections of both CA-producing and indolamine-producing neurons in the brain stem (see refs. 1 9 41 57 59 64 and others). The direct demonstration of the CA pathways to telencephalon with the GA method in the present study bridges this gap and supports the presumption that at least some of these fibre systems are too fine to be detected with the anterograde staining technique or are refractory to such staining.

The organization of the ascending CA neuron systems as revealed by the GA fluorescence method is highly complex. It reflects both the abundance of the CA fibre systems and their often complicated and interwoven courses, as well as their intricate suborganization and branching patterns. The common notion of the central CA neurons as highly diffuse and "unspecific" systems, seems to be true only in a limited sense. Thus the ascending DA neuron systems appear to have topographically highly ordered projections, and the present observations indicate that also the ascending NA fibre systems have a high degree of suborganization. This, of course has special implications in experimental [illegible]

involving lesions or stimulations of CA cell groups or fibre pathways. Thus, for example it is obvious from the organization of the projections from the locus coeruleus cell group that lesions at various points along the DTB — e g. in the locus itself in the DTB behind the tegmental radiations, in the DTB in front of the tegmental radiations, in its course through the MIB or in the cingulum — will have different denervating effects and might thus have quite different functional consequences. Also it is evident from the present observations that lesions of the so-called ventral CA bundle of Ungerstedt [41] in the rostral mesencephalon (i. e in the region of the tegmental radiations) or of the MFB system will involve several ascending CA fibre systems and will result in quite complex denervations. To overcome this and to provide possibilities for more precise experimental approaches to the different CA neuron systems, more work is necessary to clarify the suborganization of the CA pathways with respect to the origins and sites of termination of their fibres.

ACKNOWLEDGEMENTS

The authors are much indebted to Kerstin Larsson Birgit Haraldsson and Lasse Sandström for their excellent technical assistance.

The study was supported by grants from the U.S. Public Health Service (NS 06701-07) the Åke Wiberg foundation and from the Swedish Medical Research Council (No. 04X 712).

REFERENCES

1. Andén N E., A. Dahlström, K. Fuxe K. Larsson L. Olson and U Ungerstedt 1966 Ascending monoamine neurons to the telencephalon and diencephalon Acta physiol. scand., 67 313—326.
2. Ban, T., and K. Zyo 1963 Experimental studies on the mamillary peduncle and mamillotegmental tracts in the rabbit Med J Osaka Univ., *13* 241—262.
3. Bédard, P., L. Larochelle, A. Parent, and L.J Poirier 1969 The nigrostriatal pathway A correlative study based on neuroanatomical and neurochemical criteria in the cat and the monkey Exp. Neurol. *25* 365—377
4. Björklund, A. B Ehinger and B. Falck 1968 A method for differentiating dopamine from noradrenaline in tissue sections by microspectrofluorometry J Histochem. Cytochem., *16* 263—270
5. Björklund, A. B Falck, and Ch. Owman 1972 Fluorescence microscopic and microspectrofluorometric techniques for the cellular localization and characterization of biogenic amines. In Methods of Investigative and Diagnostic Endocrinology S.A. Berson, ed. Vol 1 The Thyroid and Biogenic Amines, J E. Rall and I.J Kopin eds. North-Holland Publ. Comp., Amsterdam p. 318—368
6. Björklund A., O Lindvall, R.Y Moore, and U Stenevi 1974 Adrenergic neocortical innervation. organization of dopaminergic systems from the midbrain. To be published.
7. Björklund, A., O Lindvall and L. A. Svensson 1972 Mechanisms of fluorophore formation in the histochemical glyoxylic acid method for monoamines. Histochemie *32* 113—131
8. Björklund, A., R.Y Moore A. Nobin and U Stenevi 1973 The organization of tubero-hypophyseal and reticulo-infundibular catecholamine neuron systems in the rat brain. Brain Res. *51* 171—191
9. Björklund, A. A. Nobin, and U Stenevi 1973 The use of neurotoxic dihydroxytryptamines as tools for morphological studies and localized lesioning of central indolamine neurons. Z. Zellforsch., *145* 479—501
10. Briggs, T.L., and W W Kaelber 1971 Efferent fiber connections of the dorsal and deep tegmental nuclei of Gudden. An experimental study in the cat. Brain Res. *29* 17—29
11. Bucher V.M., and S.M. Bürgi 1953 Some observations on the fiber connections of the di- and mesencephalon in the cat. Part III. The supraoptic decussations. J Comp. Neurol. *98* 355—379
12. Bürgi, S 1954 Über die zentralen Haubenbahnen. Schweiz. Med. Wschr *84* 57-61
13. Bürgi, S., und V.M. Bucher 1960 Markhaltige Faserverbindungen im Hirnstamm der Katze. Springer Verlag, Berlin-Göttingen-Heidelberg.
14. Carpenter M.B., and P Peter 1972 Nigrostriatal and nigrothalamic fibers in the rhesus monkey J Comp. Neurol, *144* 93—116.
15. Chi, C.C. 1970 An experimental silver study of the ascending projections of the central gray substance and adjacent tegmentum in the rat with observations in the cat. J Comp. Neurol. *139* 259—272.
16. Chu, N -S and F.E. Bloom 1974 The catecholamine-containing neurons in the cat dorsolateral pontine tegmentum: distribution of the cell bodies and some axonal projections. Brain Res. *66* 1—21

17 Cowan, W.M., R.W. Guillery and T.P.S. Powell 1964 The origin of the mamillary peduncle and other hypothalamic connexions from the midbrain. J Anat. Lond., *98*, 345—363
18. Crosby E.C., and R.T Woodburne 1951 The mammalian midbrain and isthmus regions. Part II. The fiber connections. J Comp. Neurol., *94* 1—32.
19 Dahlström, A., and K. Fuxe 1964 Evidence for the existence of monoamine-containing neurons in the central nervous system. I Demonstration of monoamines in the cell bodies of brain stem neurones. Acta physiol. scand., 62. Suppl. 232. 1—55
20. Forel A. 1877 Untersuchungen über die Haubenregion und ihre oberen Verknüpfungen im Gehirne des Menschen und einiger Säugethiere, mit Beiträgen zu den Methoden der Gehirnuntersuchung. Archiv f Psychiatrie *7 II* 393—495
21 Fuxe K. 1965 Evidence for the existence of monoamine neurons in the central nervous system. IV Distribution of monoamine nerve terminals in the central nervous system. Acta physiol. scand., *64* Suppl. 247 39—85
22. Fuxe, K., B. Hamberger and T Hökfelt 1968 Distribution of noradrenaline nerve terminals in cortical areas of the rat. Brain Res., *8*. 125—131
23 Fuxe, K., and Ch. Owman 1965 Cellular localization of monoamines in the area postrema of certain mammals. J Comp. Neurol., *125* 337—354
24 Golden, G.S. 1972 Embryologic demonstration of a nigro-striatal projection in the mouse. Brain Res., *44* 278—282.
25. Gurdjian, E.S. 1927 The diencephalon of the albino rat. Studies on the brain of the rat. J Comp. Neurol. *43* 1—114
26. Hamilton, B.L. 1973 Projections of the nuclei of the periaqueductal gray matter in the cat. J Comp. Neurol. *152* 45—58.
27 Hamilton B.L., and F.M. Skultety 1970 Efferent connections of the periaqueductal gray matter in the cat. J Comp. Neurol. *139* 105—114
28. Hökfelt, T., K. Fuxe O Johansson, and A. Ljungdahl 1974 Pharmaco-histochemical evidence of the existence of dopamine nerve terminals in the limbic cortex. Europ. J Pharmacol., *25* 108—112.
29 Jacobowitz, D.M. 1973 Effects of 6-hydroxydopa. In. Frontiers in Catecholamine Research. E. Usdin and S. Snyder eds. Pergamon Press, Oxford, pp. 729—739
30. Krieg, W.J.S. 1932 The hypothalamus of the albino rat. J Comp. Neurol., *55* 19—89
31 Kuru, M. and H. Yamamoto 1964 Fiber connections of the pontine detrusor nucleus (Barrington). J Comp. Neurol. *123* 161—186.
32. Kuypers, H.G.J.M 1958 Certain fiber connections of the mesencephalic central gray matter In *Reticular Formation of the Brain.* (*Eds. H.H. Jasper L.D* Proctor R.S. Knighton, W C. Noshay and R.T Costello). Little, Brown and Co., Boston, pp. 264—272.
33 König, J.F.R., and R.A. Klippel 1963 The rat brain. a stereotaxic atlas of the forebrain and lower parts of the brain stem. Williams and Wilkins Co. Baltimore.
34 Lidbrink, P., G Jonsson, and K. Fuxe 1974 Selective reserpine-resistant accumulation of catecholamines in central dopamine neurons after DOPA administration. Brain Res. *67* 439—456.
35. Lindvall, O., and A. Björklund 1974 The glyoxylic acid fluorescence histochemical method. a detailed account of the methodology for the visualization of central catecholamine neurons. Histochemistry *39* 97—127
36. Lindvall, O., A. Björklund and R.Y Moore 1974 The adrenergic innervation of

the neocortex as revealed by the glyoxylic acid fluorescence method. To be published.
37 Lindvall, O A. Björklund, A. Nobin, and U Stenevi 1974 The adrenergic innervation of the rat thalamus as revealed by the glyoxylic acid fluorescence method. J Comp. Neurol., *154* 317—348.
38. Lindvall, O., A. Björklund, and L.-A. Svensson 1974 Fluorophore formation from catecholamines and related compounds in the glyoxylic acid fluorescence histochemical method. Histochemistry in press.
39 Loizou, L.A. 1969 Projections of the nucleus locus coeruleus in the albino rat. Brain Res. *15* 563—566.
40. Lynch G R.L. Smith and R. Robertson 1973 Direct projections from brainstem to telencephalon. Exp. Brain Res., *17* 221—228.
41 Maeda, T., C. Pin, D Salvert, M. Ligier et M. Jouvet 1973 Les neurones contenant des catécholamines du tegmentum pontique et leurs voies de projection chez le chat. Brain Res., *57* 119—152.
42. Maeda, T., and N Shimizu 1972 Projections ascendantes du locus coeruleus et d'autres neurones aminergiques pontiques au niveau du prosencephale du rat. Brain Res., *36* 19—36.
43 Maler L., H.C. Fibiger and P.L. McGeer 1973 Demonstration of the nigrostriatal projection by silver staining after nigral injections of 6-hydroxydopamine Exp. Neurol., *40* 505—515
44 Mettler F.A. 1944 The tegmento-olivary and central tegmental fasciculi. J Comp. Neurol., *80* 149—175
45. Minderhoud, J.M. 1967 Observations on the supra-optic decussations in the albino rat. J Comp. Neurol. *129* 297—312.
46. Moore, R.Y 1970 Brain lesions and amine metabolism. Int. Rev Neurobiol. *13* 67—91.
47 Moore, R.Y., R.K. Bhatnagar and A. Heller 1971 Anatomical and chemical studies of a nigro-neostriatal projection in the cat. Brain Res., *30* 119—135
48. Morest, D.K. 1961 Connexions of the dorsal tegmental nucleus in rat and rabbit. J Anat. (Lond.), *95* 229—246.
49 Nauta, W.J.H. 1958 Hippocampal projections and related neural pathways to the mid-brain in the cat. Brain, *81* 319—347
50 Nauta, W.J.H. 1963 Central nervous organization and the endocrine motor system. In. Advances in Neuroendocrinology (Ed. A.V Nalbandov) Univ of Illinois Press, Urbana, pp. 5—21
51 Nauta, W.J.H., and W Haymaker 1969 Hypothalamic nuclei and fiber connections. In. The Hypothalamus. W Haymaker E. Anderson and W.J.H. Nauta eds. Charles C. Thomas, Springfield, p. 136—209
52. Nauta, W.J.H., and H.G.J.M. Kuypers 1958 Some ascending pathways in the brain stem reticular formation. In. Reticular Formation of the Brain. H.H. Jasper L.D Proctor R.S. Knighton, W C. Noshay and R.T Costello eds. Little, Brown and Co., Boston, pp. 3—30.
53. Nobin, A., and A. Björklund 1973 Topography of the monoamine neuron systems in the human brain as revealed in fetuses. Acta physiol. scand. Suppl. 388 1—40.
54. Olson, L., and K. Fuxe 1971 On the projections from the locus coeruleus noradrenaline neurons: The cerebellar innervation. Brain Res. *28* 165—171
55 Olson, L., and K. Fuxe 1972 Further mapping out of central noradrenaline neuron systems: Projections of the subcoeruleus area. Brain Res. *43* 289—295

56 Russel, G.V. 1954 The dorsal trigemino-thalamic tract in the cat reconsidered as a lateral reticulo-thalamic system of connections. J. Comp. Neurol., *101* 237—264
57 Sachs, Ch., G. Jonsson and K. Fuxe 1973 Mapping of central noradrenaline pathways with 6-hydroxy DOPA. Brain Res., *63* 49— 61
58. Scheibel, M.E., and A.B. Scheibel 1958 Structural substrates for integrative patterns in the brain stem reticular core. In: Jasper et al. Reticular formation of the brain. Little, Brown and Co. Boston. pp. 31—55
59 Segal, M., V. Pickel and F. Bloom 1973 The projections of the nucleus locus coeruleus: an autoradiographic study. Life Sci. *13* 817—821
60 Shimizu, N. and S. Ohnishi 1973 Demonstration of nigro-neostriatal tract by degeneration silver method. Exp. Brain Res. *17* 133—138
61 Szentágothai, J., B. Flerkó, B. Mess and B. Halász 1968 Hypothalamic control of the anterior pituitary. An experimental-morphological study. Akadémiai Kiadó Budapest, pp. 58—62.
62 Thierry, A.M., G. Blanc, A. Sobel, L. Stinus and J. Glowinski 1973 Dopaminergic terminals in the rat cortex. Science *182* 499—501
63 Thierry, A.M., L. Stinus, G. Blanc and J. Glowinski 1973 Some evidence for the existence of dopaminergic neurons in the rat cortex. Brain Res. *50* 230—234.
64 Ungerstedt, U. 1971 Stereotaxic mapping of the monoamine pathways in the rat brain. Acta physiol. scand. Suppl. 367 1—48
65 Valverde, F. 1965 Studies on the piriform lobe. Harvard University Press, Cambridge, Mass.
66. Wenschedel, E. 1937 Die zentrale Haubenbahn und ihre Bedeutung für das extrapyramidal-motorische System. Arch. Psych. *107* 443—579
67 Würscher, W., W. Schober and L. Werner 1965 Architektonischer Atlas vom Hirnstamm der Ratte. S. Hirzel Verlag, Leipzig.
68. Zeman, W., and J.R.M. Innes 1963 Craigie's neuroanatomy of the rat. Academic *Press, New York and London*
69 Zyo, K., T. Oki, and T. Ban 1963 Experimental studies on the medial forebrain bundle, medial longitudinal fasciculus and supraoptic decussations in the rabbit. Med. J. Osaka Univ., *13* 193—230.

ROLE OF EN

HIST

A study on

by

Curt .

CONTEN S

I T ODUCTION 6

METHODS 7

1 Isola ion of rat mast cells 7
2 Analytical procedures 7
3 Statistical method 9

RESULT AND DISCU SION 9

1 Energy metabolism in isolated mast cells 9
2 Energy dependen e of his amine release
nduced by exogenou ATP 13
3 Energy dependence of histamine release
induced by compound 48/80 15
4 Utilization of endogenous ATP during hi
stamine release 17
5 Comparison of histamine release from
mast cells with other secretory systems 19

SUMMARY 23

ACKNOWLEDGEMENTS 25

REFERENCES 26

Acta Physiologica Scandinavica
Supplementum 413

From the Department of Pharmacology Karolinska Institutet
S-104 01 Stockholm 60 Sweden

ROLE OF ENERGY METABOLISM IN HISTAMINE RELEASE

A study on isolated rat mast cells

by

Curt Peterson

Stockholm, 1974

The present survey is based on studies carried out a the Department of Pharmacology Karolinska Institutet tock holm Apart from some hitherto unpublished results the studies have been presented in the following papers

I Diamant B and C Peterson The metabolism of mono saccharides in isolated rat mast cells and its influ ence on histamine release induced by adenosine 5 triphosphate Acta physiol scand 1971 83 324 334

II Peterson C and B Diamant Inhibitory action of exogenous adenosine 5 triphosphate on glycolysis in isolated rat mast cells Significance for histamine release *Acta pharmacol et toxicol* 1974 34 Pre print

III Peterson C Inhibitory action of antimycin A on histamine release from isolated rat mast cells Acta pharmacol et toxicol 1974 34 Preprint

IV Peterson C Histamine release induced by compound 48/80 from isolated rat mast cells Dependence on endogenous ATP Acta pharmacol et toxicol 1974 34 Preprint

V Peterson C and B Diamant Increased utilization of endogenous ATP in isolated rat mast cells during hi stamine release induced by compound 48/80 Acta pharmacol et toxicol 1974 34 Preprint

These papers will be referred to by their Roman numerals

INTRODUCTION

The observation that antigen caused release of histamine from sensitized animals or tissues (Dragstedt and Gebauer Fuelnegg 1932 Bartosch Feldberg and Nagel 1932) suggested that histamine release is of importance in the pathogenesis of hypersensitivity reactions Evidence for a role of energy metabolism in histamine release was first presented by Parrot (1942) who found that anoxia inhibited anaphylactic histamine release in guinea pig tissues Combined biochemical and morphological studies indicated that histamine is present in high concentration in mast cells (Riley and West 1953 Fawcett 1954) This was confirmed when mast cells from the peritoneal cavity of the rat were concentrated by differential centrifugation (Padawa and Gordon 1955 Archer 1958) Fractionation of homogenized tissues demonstrated that histamine is stored in metachromatic granules (Mota et al 1954 West 1955)

A number of substances of relatively simple chemical structure have been found to cause extrusion of granules and histamine release from mast cells (see Paton 1957 Rothschild 1966) The histamine releasing property of compound 48/80 a condensation product of p methoxyphenethylmethylamine and formaldehyde (Baltzly et al 1949) was originally demonstrated by Paton (1951) In the rat the histamine release process evoked by compound 48/80 has many characteristics (dependence on pH temperature etc) in common with anaphylactic histamine release (Högberg and Uvnäs 1960 Mota and Ishii 1960) In glucose free media the granule extrusion and histamine release were abolished by metabolic inhibitors (Junqueira and Beiguelman 1955 Högberg and Uvnäs 1957 1960 Mota and Ishii 1960) Histamine release induced by these agents was not accompanied by a loss of cytoplasmatic constituents (Diamant 1967a Johnson and Moran 1969) On the other hand substances such as long chain alkylamines and chlorpromazine caused degranulation of mast cells and histamine release

which was not inhibited by metabolic inhibitors (Mota 1959 Högberg and Uvnäs 1960 Frisk Holmberg 1971) These agents also caused considerable loss of potassium and lactic de hydrogenase indicating a loss of cell integrity (Paton 1956 Frisk Holmberg 1971)

Glucose was found to counteract the inhibitory action of anoxia cyanide or dinitrophenol on histamine release from rat tissues induced by compound 48/80 or antigen anti body reaction (Diamant and Uvnäs 1961 Provoust Danon and Moussatché 1961 Rothschild Vugman and Rocha e Silva 1961 Diamant 1962b) This effect of glucose could be abolished by phlorizin an inhibitor of glucose transport (Diamant 1962a) From these and other studies it has been concluded that histamine release from rat mast cells in duced by compound 48/80 or antigen antibody reaction is an energy dependent reaction which can be suppo ted by oxi dative or glycolytic energy yielding reactions

In view of the indirect evidence for the involvement of cellular ATP in the histamine release induced by compound 48/80 or antigen antibody reaction the effect of exoge nous ATP on isolated rat peritoneal mast cells has been studied (Diamant and Krüger 1967 Sugiyama and Yamasaki 1969) It was found that ATP induced histamine release provided calcium was present However the histamine re leasing ability of exogenous ATP does not seem to be due to its possible role as an energy donor since a number of inhibitors of cellular energy production were found to block the release (Diamant and Krüger 1967 Sugiyama and Yamasaki 1969 Diamant and Peterson 1970 Sugiyama 1971a) Glucose did not restore histamine release induced by exo genous ATP under anaerobic conditions (Diamant and Krüger 1967) However in the absence of metabolic inhibitors glucose enhanced histamine release induced by exogenous ATP but not by compound 48/80 (Diamant and Peterson 1970)

The present studies were undertaken in order to investi gate the role of energy metabolism in histamine release from rat mast cells The aims were

1 To compare the histamine release processes induced b exogenous ATP and compound 48/80 with regard to h energy dependence

2 To correlate the ability of the mast cells to r l histamine when exposed to compound 48/80 with t tent and turnover of endogenous ATP in the cel

3 To study the energy metabolism in the mast ce l histamine release to establish whether or not th gy dependence reflects an increased utilizatio f dogenous ATP

METHODS

The methods used have been described in detail in the in dividual papers and therefore only a summary will be g en below

1 Isolation of rat mast cells

Mast cells were isolated from the peritoneal and pleural cavities of male Sprague Dawley rats following essentially the method of Uvnäs and Thon (1959 1961 Thon and Uvnäs 1966) The cell suspension obtained contained 90 95% mast cells In each experiment at least two different aliquots were counted in a Bürker chamber to determine the concen tration of mast cells (500 2000 cells counted)

2 Analytical procedures

Histamine was assayed fluorometrically after condensation with phthaldialdehyde (OPT) as described by Shore Burk halter and Cohn (1959) The extraction procedure was o- mitted since histamine was the only substance present rat mast cells which gave fluorescence in the pres

OPT (Bergendorff and Uvnäs 1972) Histamine release has been expressed as a percentage of the total histamine con tent of the cells

Analyses of ATP glucose and lactate were performed accor ding to the methods developed by Lowry and co workers (see Lowry and Passonneau 1972) These methods are based on measurements of the native fluorescence of the reduced forms of the pyridine nucleotides In the presence of the appropriate enzyme(s) the particular substrate is oxidized by NAD or NADP (or reduced by NADH or NADPH) whereby the pyridine nucleotide itself is reduced (or oxidized) When ATP was determined the fluorescence of NADPH was increased by oxidation in a strongly alkaline medium Creatine phos phate was determined as ATP after the addition of creatine kinase Glycogen was determined as glucose after boiling the samples in HCl The fluorescence of NADH or NADPH (or histamine OPT) was determined in a Farrand Ratio Fluoro meter with appropriate filters for measuring the fluore scence at 460 nm after excitation at 366 nm The assays were based on two readings made before and after the addi tion of the samples (for determination of ATP or lactate) or an auxiliary enzyme (hexokinase for determination of glucose) to the reagent solution in the fluorometer tubes The concentration of ATP glucose or lactate in the cell samples was determined by comparison with identically treated standards In some experiments internal standards were also run

The concentration of ATP creatine phosphate glucose gly ogen or lactate in the cell samples has been expressed as mmoles/kg mast cells (dry weight) The dry weight of the mast cells was determined from the cell count and the mean dry weight of single peritoneal mast cells (0 5 ng/ cell Diamant and Lowry 1966 Diamant and Glick 1967) The ATP content (per cell) in the contaminating cells was about half of that in the mast cells (Johnson and Moran 1969) The lactate concentration in suspensions of mixed perito neal cells was less than 1/3 of that in suspensions of isolated mast cells (same total cell concentration) before

incubation as well as after incubation with c
glucose and antimycin A Since the cell susp n
contained 90 95% mast cells no correction h t
for the contribution from contaminating cells

3 Statistical methods

Mean values and standard errors of the mean
ted in experimental series of at least 4 exp
series of 3 experiments ranges have been g ver
tistical significance of differences betwe n m
was tested with the Student s t test The le c
ficance is denoted by p values

RESULTS AND DISCUSSION

1 Energy metabolism in isolated mast cells

a) Endogenous substrates

Mast cells seem to have a good supply of endogenous substrates Thus during incubation at 37^0 in a glucose free medium the oxygen consumption remained unchanged for at least 4 hours (Chakravarty and Zeuthen 1965) and the ATP content was not reduced within 1 hour (Diamant 1967b) The lactate content in the cell suspensions was 5 0 0 46 mmoles/kg dry weight (mean value $^+$ S E M n 33) immediately after isolation and it did not change during incubation in a glucose free medium at 37^0 for 15 min (I II IV) The glycogen content immediately after isolation was about 5 mmoles of glycosyl units/kg dry weight

Only traces of glucose and glucose 6 phosphate were found From the oxygen consumption data given by Chakravarty and Zeuthen (1965) it can be calculated that the glycogen present would be consumed in about 1 hour if the cells only utilized glycogen This suggests that the mast cells also utilize other endogenous substrates e g lipids

b) Phosphagens

The ATP content in suspensions of untreated mast cells amounted to 1 59 $\pm$ 0 08 mmoles/kg dry weight (mean value $\pm$ S E M n 31) (III IV V) This value agrees well with those found by Diamant (1967b) and Bergqvist Samuelsson and Uvnäs (1972) but is somewhat less than the values reported by Johnson and Moran (1969) and Johansen and Chakravarty (1972) The mast cells contained much less creatine phosphate than ATP (Fig 1) which is quite the reverse of what has been found in muscle tissue (see Karlsson 1971) As a rule only ATP was measured

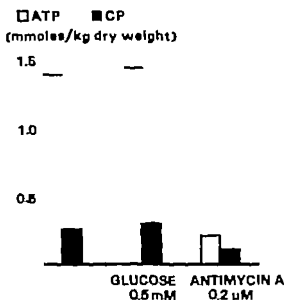


Fig 1

Contents of ATP and creatine phosphate (CP) in rat mast cells The cells were incubated for 6 min (at 37^{0}) in the presence or absence of glucose (0 5 mM) or antimycin A (0 2 µM) Mean values from 2 experiments are shown

c) Metabolism of glucose

The addition of glucose to suspensions of mast cells caused an accumulation of lactate which was related to the glu cose concentration up to about 0 5 mM (I IV) Using ^{14}C glucose Ho Cabut and Meng (1970) *found* that the lactate formation increased with the glucose concentration above 1 mM The discrepancy might be ascribed to the longer in cubation periods used by these authors (1 2 hours compared to 15 min in the present studies) The accumulation of lactate was linear with time for at least 30 min (II) and the maximal rate under aerobic conditions was about 1 mmol x kg dry weight ^{1} x min ^{1} This is in good agreement with the lactate production rate calculated indirectly by Chakravarty (1966) when mast cells were incubated in a *bicarbonate medium containing 5 mM glucose The accumu* lation of lactate accounted for 60 75% of the glucose utilization (IV) which is in good agreement with the re sults of Ho Cabut and Meng (1970)

Glucose (0 5 mM) caused a slight increase in the ATP con tent of the mast cells (IV) This can be attributed to the enhanced glycolytic flux and also to enhanced oxida tive phosphorylation since glucose elevated the oxygen consumption of mast cells (Chakravarty and Zeuthen 1965) No additional rise in ATP content was observed when the glucose concentration was further increased

d) Metabolism of other monosaccharides

Mannose (0 5 mM) was metabolized to lactate by the mast cells to the same extent as was glucose whereas no lac tate accumulation was observed on incubation with fruc tose galactose or L glucose (0 5 mM of each) (I) Chakra varty (1968) found that in addition to glucose fructose and galactose (5 mM) were also oxidized by rat mast cells However no lactate accumulation occurred when the cells were incubated with 5 mM fructose or galactose even under anae obic conditions This indicates that fructose and galactose are not metabolized to a significant rate by the glycolytic pathway in rat mast cells A similar

pattern of metabolism of monosaccharides has been found in the pancreatic islets of the rat (Jarret and Keen 1968) and the mouse (Hellerström 1967 Ashcroft Weerasinghe and Randle 1973)

e) Effect of metabolic inhibitors

Iodoacetate used as an inhibitor of glycolysis (Webb 1966) abolished the lactate accumulation from glucose but did not affect the lactate content in the absence of exogenous substrates (I)

Antimycin A which is known to inhibit electron transport in the mitochondrial respiratory chain (Wainio 1970 Slater 1973) caused lactate to accumulate from endogenous substrates (I) The accumulation rate was 0 17 mmoles x kg dry weight ^{1} x min ^{1} Antimycin A also enhanced lactate accumulation from glucose (I II) The accumulation in creased with the glucose concentration up to 1 1 mM (IV) at which concentration the accumulation rate amounted to about 3 mmoles x kg dry weight ^{1} x min ^{1} These results are at variance with those of Chakravarty (1965) who found no significant difference between the rates of ana erobic glycolysis in the presence and absence of glucose (5 mM) Our results agree with the view that the resto ring effect of glucose on histamine release induced by compound 48/80 or antigen antibody reaction is due to an increased glycolytic energy yield (Diamant 1962b)

The utilization of glucose was increased by antimycin A (IV) confirming the suggestion of a Pasteur effect in the mast cells (Chakravarty 1965) In the presence of antimycin A lactate accumulation accounted for 82 97% of the glucose utilization (IV) as compared to 60 70% in its ab ence

Antimycin A reduced the cellular ATP content to about 30% of the original value within 3 min (III) which is in good agreement with observations on the reduction of the ATP content in mouse pancreatic islets after the addition of an uncoupling agent (Ashcroft Weerasinghe and Randle

1973) The oxidative ATP generation in mast cells seems to be completely blocked by 0 2 μM antimycin A since pyruvate (4 7 mM) had no restoring effect on the ATP content (IV) In contrast glucose in only one tenth of the pyruvate concentration restored the ATP content to 70 80% of the original value Furthermore antimycin A (0 2 μM) has been shown to abolish the $^{14}CO_2$ production from 2 ^{14}C pyruvate in mast cells (Diamant and Norn unpublished observation) It should be pointed out that 0 03 μM antimycin A has been found to completely inhibit the respiration of cultured lymphoma cells (Gregg Machinist and Currie 1968)

In the light of these observations it seems reasonable to assume that the rate of lactate accumulation in suspensions of mast cells in the presence of antimycin A (0 2 μM) gives a reliable measure of the cellular ATP production From the rate of lactate accumulation after the addition of various concentrations of glucose to antimycin A treated cells it was calculated that the turnover time of ATP in the cells was about 30 sec irrespective of the steady state level (IV) This indicates that the rate of utilization of ATP in mast cells is directly proportional to the ATP content

2 Energy dependence of histamine release induced by exogenous ATP

a) Inhibition of histamine release by antimycin A

Histamine release induced by exogenous ATP and compound 48/80 showed the same sensitivity towards the inhibitory action of antimycin A (III) It was not necessary to preincubate the mast cells with antimycin A to block ATP induced histamine release as was the case when compound 48/80 was used This can be explained by the fact that histamine release caused by ATP is a relatively slow process with an initial lag period of about 1 min (Diamant and Peterson 1970 Sugiyama 1971a) whereas 48/80 induced histamine release is completed within 10 sec (Bloom Fred

holm and Haegermark 1967 Sugiyama 1971a) The length of the lag period was reduced by increasing the concentration of ATP (Dahlquist and Diamant 1974) This finding may ex plain the incomplete inhibition of histamine release ob served when concentrated mast cell suspensions were ex posed simultaneously to antimycin A and high concentrations of ATP (II)

b) Failure of glycolytic substrates to restore histamine release under anaerobic conditions

Glucose did not restore ATP induced histamine release in the presence of inhibitors of oxidative metabolism (Dia mant and Krüger 1967 I II) Mannose was also ineffective in that respect This finding can be explained by the observation that exogenous ATP but not compound 48/80 inhibited the glycolytic metabolism of glucose in rat mast cells (II) imilarly the metabolism of mannose was inhi bited The mechanism behind this action is not known It could be attributed to an inhibition of some glycolytic reaction(s) or to a reduced uptake of monosaccharides

c) Enhancement of histamine release by glycolytic substrates under aerobic conditions

Under aerobic conditions glucose and mannose i e those monosaccharides which were metabolized to lactate in the mast cells enhanced histamine release induced by exoge nous ATP but not by compound 48/80 (Diamant and Peterson 1970 I) Lactate and pyruvate also enhanced the release (I) The enhancing effect of glucose but not that of lac tate or pyruvate was abolished by 0 1 mM iodoacetate which caused a partial inhibition of the histamine release in the absence of exogenous substrates (Diamant and Peter son 1970 I) The results indicate that in the absence of exogenous substrates the energy production rate is a li miting factor for histamine release induced by exogenous ATP because of the inhibitory action of ATP on glycolysis in the mast cells However since the inhibition was not complete it seems likely that the addition of glucose

[illegible]
[illegible]
energy [illegible]
[illegible] removed

[illegible] release [illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]
[illegible]

Conclusion: The [illegible]
[illegible] release induced by [illegible]
[illegible] energy dependence [illegible]
The observed differences [illegible]
[illegible] release induced by [illegible]
[illegible]
[illegible] cells

[illegible] Energy dependence of [illegible] release
[illegible]

[illegible] release [illegible]

[illegible] release induced by [illegible]
[illegible]
[illegible]
[illegible]
[illegible]
of the release process [illegible] Fresco [illegible] and [illegible]
[illegible]

The reduction [illegible] release [illegible]
[illegible]
[illegible] ATP content [illegible]
[illegible] induced by [illegible] release [illegible]
[illegible] reduction of the [illegible] ATP content

has also been found after treatment with oligomycin (Johan sen and Chakravarty 1972)

It should be noted that a correlation has been found between the ATP content and the rate of glucose induced insulin release from mouse pancreatic islets after the addition of various metabolic inhibitors (Ashcroft Weerasinghe and Randle 1973)

b) Restoring effect of glycolytic substrates on histamine release from antimycin A treated cells

Monosaccharides (glucose or mannose) which were metabo lized to lactate by the mast cells restored the ability of antimycin A treated mast cells to release histamine on exposure to compound 48/80 (IV) The restoring effect oc curred concomitantly with an increase in the cellular ATP content which attained a steady state level within 2 5 min Histamine release was completely restored by 0 61 mM glucose which gave rise to a steady state level of ATP of about 75% of the original value

Conclusion The ability of rat mast cells to release hista mine when exposed to compound 48/80 is correlated to their ATP content (Fig 2) Practically no histamine can be re leased from cells in which the ATP content has been redu ced to about 25% of the original value On the other hand an ATP content of about 75% of the original level is suffi cient to give maximal histamine release

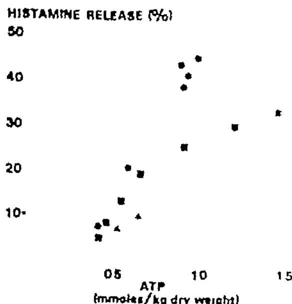


Fig 2

Histamine release induced by compound 48/80 and it ela tion to the ATP content in the mast cells The cells were treated for different times with antimycin A or gl cose and antimycin A before the addition of compound 48/80 (Re sults from Fig 3 in paper III (squares) fig 4 (circle) and fig 5 (triangles) in paper IV)

4 Utilization of endogenous ATP during histamine release

From observations on the effects of metabolic inhibitors and substrates on histamine release induced by compound 48/80 or antigen antibody reaction it has been suggested that the release process involves utilization of cellular ATP (Moussatché and Provoust Danon 1958 Chakravarty 1962 Diamant 1962b Rothschild 1963 Kahl and Wetter 1967) However Diamant (1967b) found no decrease in the ATP con tent of rat mast cells 15 sec 60 min after the addition of compound 48/80

In view of the rapid turnover of ATP in rat mast cells the question of whether or not histamine release is accompanied by an increased utilization of ATP was reinvestigated using cells in which the production of ATP has been reduced by pre incubation for 1 min with antimycin A (V) In such cells histamine release induced by compound 48/80 was accompanied by a reduction in ATP content which exceeded that in cells not exposed to the releasing agent ($p < 0\ 005$) The obser vation by Diamant (1967b) that histamine release induced by compound 48/80 from cells with an intact energy produc tion was not accompanied by a decrease in cellular ATP con tent was confirmed The results contradict the hypothesis presented by Yamasaki (1967) and upported by Sugiyama (1971b) that energy is not needed for the histamine release process <u>per se</u> According to their proposal an active energy metabolism in the mast cells is a prerequisite for the integrity and function of the cells including the abi lity to respond to a histamine releasing agent such as com- pound 48/80 A similar proposal has been made for the se cretion of vasopressin from the neurohypophysis (Thorn 1970)

Histamine release induced by chlorpromazine or n decylamine was not accompanied by a decrease in cellular ATP regard less of whether antimycin A was present or not However on prolonged incubation with these agents the ATP content de creased This might be explained by a release of cytoplas mic constituents and subsequent hydrolysis of the ATP re leased and/or by a reduced production of ATP in the cells

<u>Conclusion</u> The results indicate that histamine release induced by compound 48/80 is accompanied by an increased utilization of endogenous ATP in the mast cells This escaped detection when cells with intact metabolic pathways were used presumably because of the rapid ATP production in such cells

5 Comparison of histamine
other secretory systems

Histamine release from rat m
48/80 or antigen antibody re
in common with other secretor
tion of catecholamines from ch
medulla insulin secretion fro
and vasopressin secretion from
posterior lobe of the hypophysi
that the secretory products are s
rounded granules and morphologica
evidence have been presented which
cretion occurs by exocytosis of the n
Douglas 1968)

Histamine seems to be released from the mas l a ules as the result of a cation exchange between hi tam e ioni cally bound to a protein heparin complex the g anules and extracellular cations (Uvnäs 1964 Uvnäs and Thon 1966 Uvnäs Aborg and Bergendorff 1970) Howeve the pe centage of histamine (or serotonin) released has been reported to exceed the percentage of granules extruded (Ca lsson and Ritzén 1969 Kosál Slorach and Uvnäs 1970 Slo ach 1971) This can be explained by the observation that some granules are retained within cellular cavities in commu ication with the extracellular medium (Röhlich Ande son and Uvnäs 1971 Lagunoff 1972 Anderson Slorach and Uvnäs 1973) Although the initial course of events is believed to involve fusion of the perigranular membrane with the cell memb ane (Röh lich Anderson and Uvnäs 1971 Anderson Slorach and Uvnäs 1973 Lagunoff 1973) the proposed mechanism for histamine release seems to obviate a need for transport of all gra nules to the cell surface

Based on similarities between the excitation cont action coupling in muscle tissue (Sandow 1952) and the secreto y process the term stimulus secretion coupling has been sug gested for the sequence of events triggered by a sec eta gogue (Douglas and Rubin 1961) In addition to endogeno s

ATP calcium is believed to have an important function in this process (see Rubin 1970a) However omission of cal cium in the medium did not abolish the effect of compound 48/80 on isolated rat mast cells (Uvnäs and Thon 1961 Van Arsdel and Bray 1961 Saeki 1964 Diamant and Krüger 1967) whereas *degranulation and histamine release from tissue* mast cells were blocked (Högberg and Uvnäs 1960 Mota and Ishii 1960 Saeki 1964) The possibility remains however that intracellular depots of calcium are of importance for histamine release from isolated mast cells induced by com pound 48/80 This view is supported by the finding of Douglas and Ueda (1973) that prolonged preincubation of mast cells in the presence of EDTA abolished the release and that it was restored by the introduction of calcium

Recently it has been demonstrated that the addition of an ionophore for calcium caused histamine release and degranu lation of mast cells in a calcium containing medium (Fore man Mongar and Gomperts 1973 Cochrane and Douglas 1974) The release was accompanied by an uptake of calcium and could be inhibited by metabolic inhibitors (Foreman Mongar and Gomperts 1973) Degranulation of mast cells has also been found after intracellular injection of calcium by some authors (Kanno Cochrane and Douglas 1973) but not by others (Tasaka et al 1970)

A relationship between calcium and cyclic AMP as intracellu lar messengers in the stimulus secretion coupling has been suggested (Rasmussen 1970) In contrast to most other systems histamine release from rat mast cells induced by compound 48/80 was inhibited by dibutyryl cyclic AMP or agents sup posed to raise the cyclic AMP level (Loeffler Lovenberg and Sjoerdsma 1971) However no correlation has so far been established between the increase in cyclic AMP content in isolated rat mast cells and the inhibition of the hista mine release (Johnson Moran and Mayer 1973)

The concept that secretion is an energy dependent process is principally based on observations on the blocking action of metabolic inhibitors (cf Hokin 1951 Coore and Randle 1964 Douglas Ishida and Poisner 1965 Kirshner and Smith 1966 Rubin 1969 1970b Ball et al 1969) However some doubts have been raised concerning the direct involvement of energy metabolism (Thorn 1970)

It has been suggested that the secretion of granular material from blood platelets induced by thrombin or collagen is ac companied by an increased utilization of endogenous ATP since the decrease in intracellular ATP content exceeded the amount of ATP which was recovered extracellularly (Holmsen Day and Storm 1969 Day and Holmsen 1971) However it has been questioned if the breakdown of intracellular ATP during the secretion is connected with the secretory process (Ire land 1967) The observation that catecholamine secretion from the adrenal medulla under certain conditions was ac companied by glycogen depletion (Rubin 1969) gives indirect support for the concept that energy is utilized during se cretion

One difficulty concerned with studies on adrenal medulla and blood platelets is that most cellular ATP is stored in the amine containing granules and metabolically inactive (Falck Hillarp and Högberg 1956 Prusoff et al 1961 Stjärne 1964 Holmsen Day and Storm 1969) The observation that antimycin A did not completely deplete the ATP content in mast cells (III IV) might indicate that mast cells con tain a small pool of metabolically inactive ATP However the mast cells granules only contained traces of ATP (Berg quist Samuelsson and Uvnäs 1971) and Johnson and Moran (1969) did not find any release of ATP during histamine release induced by compound 48/80 or antigen antibody reac tion

It is not known which reaction(s) in the secretory process are dependent on energy One possibility is that energy is utilized by a contractile process It has been suggested

POISNER A M and W W DOUGLAS A possible mechanism of release of posterior pituitary hormones involving adenosine triphosphate and an adenosine triphosphatase in the neurosecretory granules Mol Pharmacol 1968 4 531 540

PROVOUST DANON A and H MOUSSATCHE Influence of anaerobic glycolysis on release of histamine in the guinea pig and rat anaphylactic reaction in vitro Nature 1961 192 361 362

PRUSOFF W H H BLASCHKO M G ORD and L A STOCKEN Incorporation of phosphorus 32 into adenosine triphosphate of adrenal chromaffin granules Nature 1961 190 354 356

RASMUSSEN H Cell communication calcium ion and cyclic adenosine monophosphate Science 1970 170 404 412

RILEY R F and G B WEST The presence of histamine in tissue mast cells J Physiol 1953 120 528 537

RÖHLICH P P ANDERSON and B UVNÄS Electron microscope observations on compound 48/80 induced degranulation in rat mast cells Evidence for sequential exocytosis of storage granules J Cell Biol 1971 51 465 483

ROTHSCHILD A M Metabolic aspects of cellular response to histamine releasing stimuli Biochem Pharmacol 1963 12 2 212

ROTHSCHILD A M Histamine release by basic compounds In Handbuch der exptl Pharmacol XVIII Ed Rocha e Silva M Springer Verlag Berlin 1966 386 430

ROTHSCHILD A M I VUGMAN and M ROCHA e SILVA Metabolic studies on the release of histamine by compound 48/80 in the rat diaphragm Biochem Pharmacol 1961 7 248 255

RUBIN R P The metabolic requirements for catecholamine release from the adrenal medulla J Physiol 1969 202 197 209

RUBIN R P The role of calcium in the release of neurotransmitter substances and hormones Pharm Rev 1970a 22 389 428

RUBIN R P The role of energy metabolism in calcium evoked secretion from the adrenal medulla J Physiol 1970b 206 181 192

SAEKI K Effects of compound 48/80 chymotrypsin and antiserum on isolated mast cells under aerobic and anaerobic conditions Jap J Pharmacol 1964 14 375 390

ANDOW A Excitation contraction coupling in muscular response Yale J Biol Med 1952 25 176 201

SCHAUER A and M EDER Die Entwicklung von Mucopoly saccchariden und Bilding histochemisch nachweisbarer Enzyme während der Mastzellreifung Virchows Arch path Anat Physiol 1962 335 72 83

SHORE P A A BURKHALTER and V H COHN A method for the fluorometric assay of histamine in tissues J Pharmacol exp Ther 1959 127 182 186

SLATER E C The mechanism of action of the respiratory in hibitor antimycin Biochim Biophys Acta 1973 301 129 154

SLORACH S A Histamine and heparin release from isolated rat mast cells exposed to compound 48/80 Acta physiol scand 1971 82 91 97

STJÄRNE L Studies of catecholamine uptake storage and release mechanisms Acta physiol scand 1964 Suppl 228 Thesis

SUGIYAMA K Calcium dependent histamine release with de granulation from isolated rat mast cells by adenosine 5 triphosphate Jap J Pharmacol 1971a 21 209 226

SUGIYAMA K Significance of ATP splitting activity of rat peritoneal mast cells in the histamine release induced by exogenous ATP Jap J Pharmacol 1971b 21 531 539

SUGIYAMA K and H YAMASAKI Calcium dependent histamine release by ATP from isolated rat mast cells Jap J Pharmacol 1969 19 175 176

TASAKA K K SUGIYAMA S KOMOTO and H YAMASAKI Degra nulation of isolated rat mast cells induced by adenosine triphosphate in the presence of calcium ions Proc Jap Acad 1970 46 317 321

THON I L and B UVNÄS Mode of storage of histamine in mast cells Acta physiol scand 1966 67 455 470

THORN N A Mechanism of release of neurohypophyseal hor mones In Aspects of neuroendocrinology Eds W Barg mann and B Scharrer Springer Verlag Berlin 1970 140 152

TRIFARO J M B COLLIER A LASTOWECKA and B STERN Inhibition by colchicin and by vinblastine of acetyl choline induced catecholamine release from the adrenal gland an anticholinergic action not an effect upon microtubules Mol Pharmacol 1972 8 264 267

UVNÄS B Release processes in mast cells and their acti vation by injury Ann N Y Acad Sci 1964 116 880 890

UVNÄS B C H ABORG and A BERGENDORFF Storage of histamine in mast cells Evidence for an ionic binding of histamine to protein carboxyls in the granule heparin protein complex Acta physiol scand 1970 Suppl 336

UVNÄS B and I L THON Isolation of biologically intact mast cells Exp Cell Res 1959 18 512 520

UVNÄS B and I L THON Evidence for enzymatic histamine release from isolated rat mast cells Exp Cell Res 1961 23 45 57

UVNÄS B and I L THON A physico chemical model of hi stamine release from mast cells In Mechanisms of Re lease of Biogenic Amines Eds U S von Euler S Rosell and B Uvnäs Pergamon Press 1966 361 370

VAN ARSDEL P P and R E BRAY On the release of histamine from rat peritoneal mast cells by compound 48/80 Effects of metabolic inhibitors J Pharmacol exp Ther 1961 133 319 324

WAINIO W W In The Mammalian Mitochondrial Respiratory Chain Academic Press 1970 112 121

WEBB J L In Enzyme and Metabolic Inhibitors Academic Press 1966 III 1 283

WESSELS N K B S SPOONER J F ASH M O BRADLEY M A LUDUENA E L TAYLOR J T WRENN and K M YAMADA Micro filaments in cellular and developmented processes Science 1971 171 135 143

WEST G B Histamine in mast cell granules J Pharm Pharmacol 1955 7 80

WOODIN A M and A A WIENEKE The participation of calci um adenosine triphosphate and adenosine triphosphatase in the extrusion of the granule proteins from the poly morphonuclear leucocyte Biochem J 1964 90 498 509

YAMASAKI H The release of histamine and its mechanism Proc 17th Jap Med Congr 1967 555 578

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 414

From the Departments of Aviation and Naval Medicine
Faculty of Medicine, Karolinska Institutet,
Stockholm, Sweden

Cardiorespiratory and Metabolic Functions During Exercise in the Hyperbaric Environment

BY

LENNART FAGRAEUS

STOCKHOLM 1974

CONTENTS

PREFACE 5

Symbols and abbreviations 6

INTRODUCTION 7

METHODS and PROCEDURES. 10

Subjects 10
Compression chamber and breathing system. 10
Exercise tests 12
Methods 13
Procedures 14
Analysis of data 15

RESULTS 16

A. Submaximal work 16
B. Maximal work 19

DISCUSSION 22

Ventilation and CO_2 elimination 22
Oxygen uptake 24
Heart rate 26
Point of exhaustion 28

GENERAL SUMMARY 31

A. Submaximal work 31
B Maximal work 32

REFERENCES 34

The present publication is based on studies reported in the following papers

I. Fagraeus L, C M Hesser and D Linnarsson Cardiorespiratory responses to graded exercise at increased ambient air pressure Acta physiol scand In press

II. Fagraeus L, J Karlsson, D Linnarsson and B Saltin Oxygen uptake during maximal work at lowered and raised ambient air pressures Acta physiol scand 1973 87 411-421

III. Linnarsson D, J Karlsson, L Fagraeus and B Saltin Muscle metabolites and oxygen deficit with exercise in hypoxia and hyperoxia J appl Physiol 1974 36 399-40?

IV. Fagraeus L. Maximal work performance at raised air and helium-oxygen pressures Acta physiol. scand. In press

V. Fagraeus L. and D Linnarsson Heart rate in hyperbaric environment after autonomic blockade Försvarsmedicin (Stockholm) 1973 9 60 261

In the text these papers will be referred to by their Roman numerals I – V

PREFACE

The present investigation was performed at the Departments of Aviation and Naval Medicine Karolinska Institutet Stockholm

To Professor Carl Magnus Hesser Head of the Department of Naval Medicine who introduced me into this field of physiology and who initiated this investigation I wish to convey my sincere and warm thanks for his continuous encouragement and never failing support and for all discussions throughout the whole investigation.

I am further much indebted to Professor Hilding Bjurstedt Head of the Department of Aviation Medicine for stimulating advice and constructive criticism based on a profound knowledge in environmental physiology

To Dr Dag Linnarsson, my close colleague and co-author I wish to express my warmest thanks for a most stimulating and enjoyable time in the laboratory valuable medical and technical assistance and fruitful discussions

To Professor Bengt Saltin and Assistant Professor Jan Karlsson, my friends and co-authors I am indebted for amicable cooperation and valuable advice

I am also deeply obliged to Gunilla Haggård and Irène Unander Scharin for having given me excellent secretarial help and to Karin Vasser and Barbro Bergström for their skilful assistance in the processing of data and the drawing of diagrams

I am very much indebted to Bertil Lindborg for his assistance in the electronic processing of primary data and to Berndt Lennholm and Bertil Lundin for their competent technical assistance both inside and outside the compression chamber and for other courtesies too numerous to mention.

The study was supported in full by the Swedish Medical Research Council (Project No 40X-682)

Stockholm April 1974

Lennart Fagraeus

Symbols and abbreviations

The symbols and abbreviations in this investigation conform to the guidelines devised by the Glossary Committee of the International Union of Physiological Sciences (see Glossary Committee of the IUPS 1973 a b)

Primary symbols

P	Partial pressure of gas given in mm Hg at actual body temperature
V	Gas volume in general
V	Gas volume per unit time
F	Fractional concentration in dry gas phase
ATA	Atmosphere(s) absolute 1 0 ATA is equivalent to 760 mm Hg or 1 013 10^5 N m^{-} or 1 013 bar or to the pressure exerted by approx 10 0 meters of sea water

Suffixes

I	Inspired gas
E	Expired gas
T	Tidal gas
A	Alveolar gas
D	Dead space

Other symbols

STPD	Standard temperature and pressure dry ($0^{o}C$ 760 mm Hg)
ATPS	Ambient temperature and pressure saturated with water vapor
BTPS	Body temperature barometric pressure and saturated with water vapor
W	Watt 1 W = 6 1. kpm min ^{1}

Symbols used for the statistical treatment of data

n	Number of subjects or observations
Range	Smallest and greatest value observed
M	Arithmetic mean
SD	Standard deviation
SEM	Standard error of the mean
p	Probability

INTRODUCTION

It is known since ancient times that man is capable of living and working in the high-pressure environment. This environment involves changes in a number of physical factors such as partial pressures of O_2 and N_2, gas density and pressure per se, which may exert definite physiological effects. The foundation of our knowledge of these effects was laid by Paul Bert (1878). He and later Boycott, Damant and Haldane (1908) recognized that there is danger not only in remaining at pressure but also in decompression to the normal environment. It is now well established that the increased N_2 pressure during air breathing in deep diving exerts a "narcotic" influence which affects psychomotor performance (for review, see Bennett 1969) and that increased air density increases the airway resistance with a consequent reduction of the ventilatory capacity (for review, see Lanphier 1969). High oxygen pressures may exert toxic effects on the cells of the central nervous system and on the respiratory epithelium (for review, see Lambertsen 1965). Finally, it is known that high pressures per se can influence biological processes, although it is still not possible to identify the exact point where pressure exerts the first possible effect (for review, see Fenn 1969). To avoid the adverse effects of air at high pressures, gas mixtures of helium and low concentrations of oxygen have been used in diving to great depths.

Comparatively little is known about the physiological adjustments to muscular exercise that occur in the high-pressure environment. Triger (1841) and Friedberg (1872) seem to have been the first to report physiological effects of muscular work at high air pressures (cited by Hoff 1948). Triger, who used a caisson designed by him for a working depth of 20 meters, i.e. for a pressure of 3.0 atmospheres absolute (ATA), reported that laborers claimed that when ascending the ladder from the working chamber while still under pressure, they felt much less out of breath than when performing similar exertion

in the open air Friedberg in reviewing the historical development of compressed air work, stated that there was acceleration of the metabolic processes and that workers claimed that they had greater muscular power while subjected to high pressure

In spite of the fact that air has so far been and probably always will be the breathing gas of choice in caisson work and in diving operations to moderate depths relatively few investigations have been undertaken to study the physiological adjustments to muscular exercise during acute exposure to hyperbaric air Ventilatory responses to submaximal exercise at increased air pressures have been studied by i e Lamphier (1963) Jarrett (1966) Taunton et al (1970) Miller Wangensteen and Lanphier (1971 1972) Wood and Bryan (1971) Lally Moore and Hong (1971) and Broussolle et al (1972) There is general agreement that ventilation becomes depressed when the air pressure is raised above normal and also that this depression at least in part is related to the increase in gas density Secondary to the inadequate ventilatory response to exercise in hyperbaric air alveolar P_{CO_2} may rise considerably This increase in alveolar P_{CO_2} is more marked in trained divers than in subjects who have not previously been exposed to raised air pressures (Lamphier 1963 Jarrett 1966 Broussolle et al 1972) The oxygen uptake (V_{O_2}) during heavy exercise at 2 0 ATA air was measured in two subjects by Taunton et al (1970) who observed an average increase in V_{O_2} of about 14 % as compared to 1 0 ATA air This increase in V_{O_2} was ascribed in part to the increased work of breathing a denser gas and in part to an increased efficiency of oxygen utilization at 2 0 ATA air On the other hand Lally et al *(1971) observed no increase in $\dot{V}_{O_2}$ during light to moderate* exercise at 2 0 ATA air Heart rate during light exercise in hyperbaric air was studied by Shilling Hawkins and Hansen (1936) who found a progressive decrease in exercise heart rate when the ambient air pressure was raised stepwise to 10 0 ATA They attributed this relative bradycardia to the increase in O_2 pressure

*Little is known about the physiology of maximal exercise in the hyper*baric environment At the moderately raised air pressures of 1 14 ATA

(Wyndham et al 1970) and 1 19 ATA (Eagan and Plese 1969) maximal oxygen uptake has been found to be significantly increased this change being ascribed by Wyndham et al to the increase in oxygen pressure However when the oxygen pressure was raised to 3 0 ATA Kaijser (1970) observed no changes in maximal oxygen uptake and performance time When the inspired oxygen pressure was kept constant a rise in the N_2 pressure up to approximately 3 0 ATA reduced the maximal oxygen uptake by 32 %, and ventilation by 62 % (Cook 1970) At 3 0 ATA air Varène Jacquemin and L'Huiller (1972) found no consistent change in either oxygen uptake or ventilation during exhaustive exercise as compared to 1 0 ATA air However these authors reported that four out of five subjects were able to exercise at a higher work load in the hyperbaric condition. Whereas Eagan and Plese (1969) reported that maximal heart rate was lower than control at 1 19 ATA air Wyndham et al. (1970) and Varène et al (1972) found no significant changes in heart rate at exhaustion at 1 14 and 3 0 ATA air respectively

The objectives of the present investigation were to study the effects of acute exposure to raised ambient air pressures on the adaptation of cardio-respiratory and metabolic functions to graded submaximal and maximal exercise and furthermore to evaluate the separate effects of increases in O_2 pressure N_2 pressure gas density and pressure per se For this purpose exercise data obtained on subjects breathing air at raised pressures in the range 1 0 6 0 ATA were compared to those obtained on the same subjects breathing oxygen at 1.0 and 1.3 ATA or a mixture of 21 % O_2 in helium at 3 0 ATA.

METHODS AND PROCEDURES

Descriptions of the methods and procedures used in this investigation have been given in detail in papers I - V and are therefore only briefly summarized below The experimental conditions to which the subjects were exposed in the separate studies are presented in Table I

Subjects

The experiments were performed on 21 healthy volunteers 10 of which participated in more than one study They were all physically active and some were well-trained in endurance sports All subjects were familiar with exposure to raised ambient pressures seven were professional Navy divers Those subjects who participated in the maximal exercise tests were all well acquainted with the sensations experienced in exhaustive exercise Anthropometric and functional data for the group of subjects are summarized in Table II individual data having been reported in the respective papers

Compression chamber and breathing system

All experiments were performed in a dry compression chamber with an inner diameter of 2 1 m length 3 9 m volume 1_ 6 m^3 The temperature in the chamber could be set at predetermined levels by means of a radiator-fan assembly controlled by a thermostat (Billman Sweden) In all experiments the chamber temperature was kept at 21 23 $^{\circ}C$ and the pressure controlled to within 1 mm Hg of any given preset level The experimental procedure inside the chamber was closely supervised through port holes in the chamber wall and by means of closed-circuit TV combined with an inter-communication system

The subjects were provided with gas mixtures of known and constant composition from high pressure tanks outside the chamber Inside the chamber the gas from the high pressure tank was allowed to pass either a 200 liter

TABLE I Experimental conditions in studies I V

Study	n	Inspired gas	Ambient Pressure ATA	Work Load
I	7	Air	1 0 4 5	50 100 150 W
		O	1 0	
II	7	Air	1 0 1 13 1 40 2 0 3 0	50-60 of V_{O_2}max and max exercise
III	6	Air	1 0 1 40	50 of V_{O_2}max and max exercise
IV	8	Air	1 0 3 0 6 0	100 W and max exercise
		21 4 % O_2 in helium	3 0	
V	6	Air	1 0 6 0	50 W
		O_2	1 3	

TABLE II. Anthropometric and functional data of 21 subjects

	Age yrs	Height cm	Weight kg	V_{O_2}max *) l min^{-1} STPD
Mean	28 6	180 9	73 2	3 74
SD	4 2	6 1	6 8	0 71
Range	23 36	171 189	58-81	2 16-5 40

*) In seven subjects (I) V_{O_2}max was predicted according to Åstrand (1960)

Douglas bag partially filled with water or a large water bottle to become humidified and then via wide bore tubings was led past the inspiratory side of a low resistance breathing valve (dead space 10 ml) (v Döbeln 1919) at a flow rate of about 3 5 l min^{-1} ATPS In this way since the volume of the wide bore tubing distal to the point of attachment of the breathing valve exceeded 1 5 liters no chamber air was inspired by the subject even during maximal inspiratory effort In one study (I) the subject inhaled the humidified gas in the Douglas bag via a Venturi type low resistance flowmeter (Wigertz 1969) Expired gas was collected in 300 liter Douglas bags and the volumes were measured at atmospheric pressure by means of a balanced Tissot spirometer (II III IV) The chamber was continuously vented as needed to maintain constant inside pressure

Exercise tests

All experimental data were obtained with the subjects in the sitting position at rest or performing submaximal or maximal exercise on a mechanically (I II III) or electrically (IV V) braked cycle ergometer The motor box of the latter ergometer (Elema Schönander Sweden) was continuously and slowly flushed with 100 % nitrogen to exclude fire hazard from raised O_2 pressures in the hyperbaric experiments The work loads used in the various studies (I – V) are shown in Table I In the study on graded submaximal exercise (I) the subjects exercised for 6 min at each of three consecutive loads of 50 100 and 150 W and in the study on autonomic blockade (V) 6 min periods of rest and exercise at 50 W were alternated repeatedly In the studies on maximal exercise the maximal work tests were preceded by 2 5 min of 'warm-up exercise at loads corresponding to 50 60 % of V_{O_2}max (II III) or at 100 W (IV) The work loads in the maximal exercise tests were chosen on an individual basis so as to bring the subjects to exhaustion within 3 5 min under normobaric condition, breathing air This supramaximal work level was determined on a separate day prior to the experiments and averaged for the group 1.2 % of the maximal aerobic power of the subjects as determined at 1 0 ATA breathing air By keeping the duration of the maximal exercise

tests within 3 5 min any potential risk for decompression sickness in the hyperbaric experiments was minimized

Methods

In study I pulmonary ventilation was measured by means of a Venturi -type flowmeter in combination with on-line digital computation techniques (Åström and Wigertz 1966) yielding tidal volume (V_T) respiratory frequency (f) and inspired minute volume (V_I) In the other studies (II III, IV) ventilation (V_E) was obtained from expired volumes collected in Douglas bags The composition of inspired and expired gases was analyzed on duplicate samples according to Scholander (1947) Based on the above data oxygen uptake (V_{O_2}) carbon dioxide elimination (V_{CO_2}) and ventilatory equivalents for O_2 (V_E/V_{O_2}) and carbon dioxide (V_E/V_{CO_2}) were obtained For recording of end tidal P_{CO_2} gas from the mouthpiece (IV) or from a special breath-by--breath sampler (Brismar Hesser and Matell 1962) (I) was led through the chamber wall and analyzed continuously for CO by means of external fast responding analyzers (Capnograph Godart or Model LB-I, Beckman Instruments) In one study on maximal work (II) alveolar P_{CO_2} was calculated by Bohr s equation assuming the V_D/V_T ratio to be 0 12 in exhaustive exercise (cf Asmussen and Nielsen 1956) All respiratory data in the submaximal exercise experiments were measured as time-averages over the 6th min of work at each level whereas those on maximal exercise represent the highest mean values obtained over the last two 30 s intervals in exhaustive exercise

Heart rate (HR) was recorded beat by-beat by means of chest electrodes and a linear cardiotachometer (Lindborg Wigertz and Ödman 1969) Submaximal HR values were obtained as time-averages over the 6th min of work at each load whereas maximal HR (HR_{max}) was defined as the peak value at the point of exhaustion.

Blood lactate concentration was obtained from arterialized capillary samples from the finger tip analysed according to Barker and Summerson (1941) as modified by Ström (1949) (II IV) or by an enzymatic method adapted for small blood samples (Scholtz et al 1959) (III) Samples for determination

of peak blood lactate concentration were taken 1 and 4 min after cessation of the maximal work test.

Muscle metabolites were examined by taking specimens from the lateral portion of the thigh (vastus lateralis of the quadriceps femoris muscle) (III) The muscle samples were obtained by means of a needle biopsy technique (Bergström 196) Skin and fascia were anaesthetized with 1-_ ml of Lidocain (1 %) and a short incision (5–10 mm) was made with a scalpel These pre parations were made with the subject in the supine position. Muscle specimens were taken before and immediately after cessation of submaximal and maximal exercise The specimens were frozen in liquid nitrogen within 3–4 s after the needle was withdrawn from the muscle and then stored at 80°C until analyzed for ATP CP glycogen, glucose glucose-6-phosphate pyruvate and lactate with methods described by Karlsson (1971)

Inspiratory gas flow V_T f V_I end-tidal F_{CO_2} and HR were recorded continuously and simultaneously on an 8-channel ink recorder (Brush Mark 200) A 14-channel analog (FM) magnetic tape recorder (Ampex FR 100 C) was used for storage and subsequent off line analysis of data

Procedures

In all five studies (I V) the experiments at raised pressures were carried out according to the same general procedure as used in the corresponding control situation at 1 0 ATA air except for the compression and decompression phases Following compression at least 5 min was allowed for the subject to become adjusted to the conditions and for chamber tempera ture to attain equilibrium In each experiment the subject in the chamber was accompanied by at least one assistant experimenter who carried out various tasks according to the experimental protocol in collaboration with the inves tigator outside the chamber Decompression to atmospheric pressure was accomplished on air according to standard decompression tables No symptoms of decompression sickness were observed Before each experiment routine medical examinations were made to ensure that the subjects were in good physical condition.

Analysis of data

To estimate the separate effects of raised O pressure data from the O_2 experiments at 1 0 and 1 3 ATA or from the He-O experiments at 3 0 ATA were compared to those obtained on air at 1 0 ATA (I IV V) The effects of raised N_2 pressure were disclosed by comparing data from the air experiments at 4 5 and 6 0 ATA to those obtained on O at 1 0 and 1 3 ATA respectively or by comparing the air data at 3 0 ATA to the He-O_2 data at the same pressure (I IV V) The combined effects of increased O and N_2 pressures were obtained by comparing the air data at raised ambient pres sures to those at 1 0 ATA (I V)

Conventional statistical methods have been applied for the calculation of arithmetical mean standard deviation and standard error of the mean. The statistical significance of differences between mean values were eval uated by applying the Student t-test to the intraindi idual differences (cf Fisher 1948)

RESULTS

The results are presented in two main sections dealing with the adaptation of cardiorespiratory and metabolic functions to exercise during acute exposure to hyperbaric environments with special emphasis on the separate effects of increases in inspired O_2 pressure inert gas pressure gas density and pressure *per se* The first section deals with submaximal exercise performed at different preset work loads whereas the second section presents data obtained during maximal exercise For the sake of clarity only the main results pertaining to selected variables are included For additional information the reader is referred to the detailed accounts given in the separate papers (I – V)

A Submaximal work

The values given in this section refer to the stable–state situation (6th min) of exercise performed at ergometric work loads of 50 100 and 150 W under three different ambient conditions *viz* with the subjects breathing air at 1 0 ATA oxygen at 1 0 ATA and air at 4 5 ATA (I Table III) In the case of the heart rate additional values are included for submaximal exercise (50 60 % of V_{O_2}max) at 1 13 1 40 2 0 and 3 0 ATA air (II) and for light exercise (50 W) at 1 3 ATA O_2 and 6 0 ATA air before and after autonomic blockade (V)

Oxygen uptake (V_{O_2}) increased in a linear fashion with the ergometric work load both at 1 0 and 4 5 ATA air (I Fig 1) At the highest load (150 W) V_{O_2} was 12 % greater (p 0 01) in the hyperbaric environment The regression equations relating V_{O_2} to work load (wl) in the two conditions were

$$V_{O_2} = 486 + 10\ 07\ wl \quad \text{at } 1\ 0 \text{ ATA} \quad (n = 21 \quad r = 0\ 978)$$

$$V_{O_2} = 464 + 11\ 67\ wl \quad \text{at } 4\ 5 \text{ ATA} \quad (n = 21 \quad r = 0\ 989)$$

where V_{O_2} is expressed in ml min^{-1} STPD and wl in watt

In the O_2 experiments at 1 0 ATA the exercise V_{O_2} values were assumed to be similar to those observed at 1 0 ATA air as it has been shown that substituting oxygen for air at normal atmospheric pressure has no significant influence on V_{O_2} in submaximal exercise (Asmussen and Nielsen 1955)

Carbon dioxide elimination (V_{CO_2}) also increased linearly with the ergometric load in all three conditions At all work loads V_{CO_2} was somewhat greater in the hyperbaric than in the two normobaric conditions This increase in V_{CO_2} in the high pressure condition was proportional to the increase in V_{O_2} however as indicated by the fact that the respiratory exchange ratio did not change significantly when the air pressure was raised from 1 0 to 4 5 ATA

When related to the ergometric work load the ventilatory response to graded exercise was similar in all three conditions except that the pulmonary ventilation (V_I) was significantly reduced by 9 % at the highest load when oxygen was substituted for air at 1 0 ATA That a rise in the ambient air pressure to 4 5 ATA and thus in N_2 pressure to 3 5 ATA did not affect V_I at any given load (Fig 1) would seem to indicate that high N_2 pressure had no significant influence on the ventilatory response to exercise However since V_{O_2} and thus the metabolic load was greater in the hyperbaric environment it follows that V_I was less for a given metabolic load at 4 5 ATA breathing air than at 1 0 ATA breathing air or oxygen (I Fig 2) At a V_{O_2} of 2 0 l min^{-1} the reduction in V_I due to the rise in N_2 pressure was calculated to average 3 3 l min^{-1} or approximately 8 %. That the pulmonary ventilation was reduced both by high P_{O_2} and by high P_{N_2} is also reflected by the fact that the ventilatory equivalents for oxygen (V_I/V_{O_2}) and for CO_2 (V_I/V_{CO_2}) were reduced at 100 and 150 W when oxygen was substituted for air at 1 0 ATA and further reduced when the pressure during air breathing was raised to 4 5 ATA.

End-tidal P_{CO_2} increased with the work load in all three conditions (I Fig 4) Because of the reduction in ventilatory response to 150 W during O_2 breathing at 1 0 ATA end-tidal P_{CO_2} rose significantly from a control value of 45 to 48 mm Hg The further non-O_2-dependent reduction in V_I observed when the pressure was raised to 4 5 ATA caused significant

increases in end-tidal P_{CO_2} at all three work loads At 150 W in the 4 5 ATA experiments end-tidal P_{CO_2} averaged 53 mm Hg

Heart rate (HR) increased linearly with the work load in all three conditions (I) However at a given ergometric load HR decreased consistently as the ambient air pressure was raised (I II V) *This lowering of the heart* rate was found to be more marked when HR was related to a given V_{O_2} (I) For any given V_{O_2} HR was lower during O_2 breathing at 1 0 ATA and still lower at 4 5 ATA breathing air than in the control situation (I Fig 5) The regression equations relating HR to V_{O_2} were:

HR = 48 2 + 40 * V_{O_2} at 1 0 ATA air (n = 8 r = 0 923)
HR = 47 6 + 37 0 V_{O_2} at 1 0 ATA O_2 (n = 28 r = 0 884)
HR = 30 2 + 36 6 V_{O_2} at 4 5 ATA air (n = 28 r = 0 945)

where HR is expressed in beats min^{-1} and V_{O_2} in l min^{-1} STPD

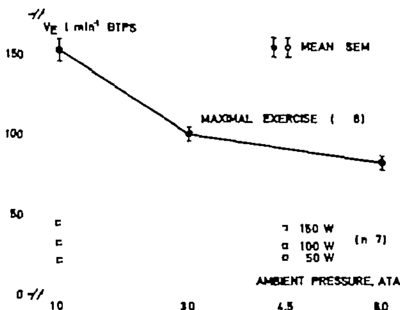


Fig 1 Steady state ventilation during submaximal (50 100 and 150 W) exercise at 1 0 and 4 5 ATA air and ventilation during the last 30 s of *exhaustive exercise at 1 0 3 0 and 6 0 ATA air (based on data from I and IV)*

The contributions of sympathetic and parasympathetic influences to the O_2- and N_2 induced decreases in HR were studied by means of differentiated autonomic blockade induced by administration of propranolol (10 mg i v) or atropine (2   2 5 mg i v) or both drugs in combination (V) In experiments with light exercise (50 W) at 1 3 ATA O_2 and 6 0 ATA air (P_{O_2} = 1 3 ATA) it was found that the O -induced reduction in HR was unaffected after propranolol and somewhat less after atropine alone or in combination with propranolol The non-O -dependent decrease in HR on the other hand remained unaffected after atropine but was significantly reduced after propranolol alone or in combination with atropine

B Maximal work

In this section respiratory heart rate and blood lactate data refer to maximal exercise breathing air in the pressure range 1 0   6 0 ATA (II III IV) and He-O_2 at 3 0 ATA (IV) Data on muscle metabolites were obtained at 1 0 and 1 40 ATA air (III) All data refer to mean values obtained over the last 30 s of exhaustive work unless otherwise stated

Oxygen uptake (V_{O_2}max   Fig 2) increased by 3 and 8 11 % respectively when the ambient pressure was increased to 1 15 and 1 40 ATA (II III) However with a further rise in pressure to 2 0 and 3 0 ATA V_{O_2}max was reduced below values at 1 40 ATA (II) and at 6 0 ATA was not significantly different from control (IV) With He-O_2 at 3 0 ATA V_{O_2}max was increased by 13 % above control values at 1 0 ATA air (IV)

Carbon dioxid elimination increased slightly as the ambient pressure was raised to 1 40 ATA and then decreased when the pressure was further raised to 2 0 ATA (II) At 3 0 ATA V_{CO_2} was actually lower than at 1 0 ATA air (II IV) At 6 0 ATA V_{CO_2} was significantly lowered by 0 49 l min^{-1} STPD below control value (IV) Inhalation of He-O_2 at 3 0 ATA however caused no consistent change in V_{CO_2} (IV)

Pulmonary ventilation (Figs 1 and 2) decreased as the ambient air pressure was raised At 1 40 ATA this decrease averaged 7 % (II) at 3 0

ATA 33 % (II IV) and at 6 0 ATA 46 % (IV) With He-O_2 at 3 0 ATA ventilation was decreased by 14 % as compared to control values at 1 0 ATA air (IV)

End-tidal P_{CO_2} (Fig 2) which averaged 33 3 mm Hg at 1 0 ATA air rose with the ambient pressure At 1 40 ATA the increase averaged 10 % and at 2 0 and 3 0 ATA 21 and 39 % respectively (II IV) At 6 0 ATA the increase amounted to no less than 63 % as compared to control (IV) At 3 0 ATA He-O_2 end-tidal P_{CO_2} was 13 % higher than at 1 0 ATA air (IV)

Heart rate at the point of exhaustion (HR_{max} Fig 3) amounted to 185 7 beats min^{-1} at 1 0 ATA and showed no consistent differences in

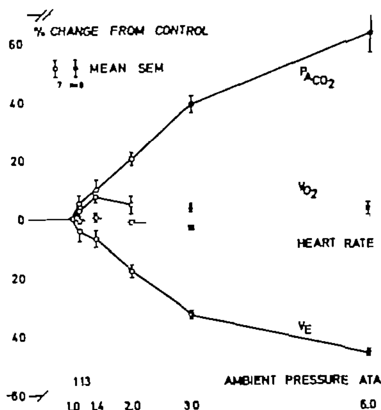


Fig 2 Cardiorespiratory adjustments in maximal exercise at increased atmospheric air pressures Data compiled from II and IV and shown on a percentile basis values at 1 0 ATA air being taken as 100 % (P_{ACO_2} values calculated or uncorrected end tidal values)

the pressure range up to 2 0 ATA (II) At 3 0 and 6 0 ATA however HR_{max} was significantly decreased by 3 and 5 % respectively as compared to control (II IV) With 3 0 ATA $He\text{-}O_2$ HR_{max} was significantly lower than at 1 0 ATA air but higher than at 3 0 ATA air (IV)

Peak blood lactate concentrations did not differ significantly between the various pressure levels (1 0 - 6 0 ATA) (II III IV) Oxygen deficit and concentrations of muscle lactate ATP CP pyruvate glucose and glucose-6-phosphate as well as glycogen depletion at exhaustion remained unchanged when the air pressure was raised to 1 40 ATA (III)

Endurance time i e the time from start of maximal exercise to exhaustion was related to the ambient pressure in a manner similar to $V_{O_2}max$ Thus endurance time was significantly prolonged by 15 0 % at 1 40 ATA (II III) but showed no further increase when the pressure was raised to 2 0 and 3 0 ATA (II) At 6 0 ATA air endurance time did not differ from control whereas at 3 0 ATA $He\text{-}O_2$ it was significantly prolonged by 14 % (IV)

DISCUSSION

Ventilation and CO_2 elimination

It is known that the ventilatory capacity is reduced in the hyperbaric air environment (for review see Lanphier 1969) and also that exercise ventilation is significantly decreased (Lanphier 1963 1967 1969 Jarrett 1966 Taunton et al 1970 Lally Moore and Hong 1971 Miller Wangensteen and Lanphier 1971 1972 Wood and Bryan 1971 Broussolle et al 1972) However no attempts have so far been made to evaluate the relative contributions of raised P_{O_2} and P_{N_2} to the reduction of the ventilatory response to exercise in hyperbaric air

In the present investigation the separate effect of raised O_2 pressure on ventilation (V_I) during graded submaximal exercise was studied by substituting O_2 for air at 1 0 ATA (I) At the highest work load (150 W) V_I decreased significantly by approximately 9 %, but showed no consistent changes at 50 and 100 W That V_I was reduced by hyperoxia in heavy but not in mild exercise is in agreement with earlier reports (cf Asmussen and Nielsen 1946 1958 Bannister and Cunningham 1954 Lambertsen et al 1959) This effect of hyperoxia has been ascribed to improved aerobic conditions in the working muscles and to withdrawal of the hypoxic ventilatory drive originating in the arterial chemoreceptors (cf Asmussen and Nielsen 1958) To evaluate the effect of raised N_2 pressure the ventilatory data from the 4 5 ATA air experiments were compared to those obtained at 1 0 ATA O_2 (I) When related to the metabolic (V_{O_2}) rather than the ergometric load V_I was found to be reduced by about 8 % at an O_2 uptake of 2 0 l min^{-1} STPD as the inspired N_2 pressure was raised to 3 5 ATA (total air pressure = 4 5 ATA) Thus at this metabolic load the shares contributed by O_2- and non-O_2-dependent factors to the total ventilatory reduction were of similar magnitude at 4 5 ATA air

That the non-O_2-dependent reduction in ventilation was due to a "narcotic" effect of the increased N_2 pressure seems unlikely in view of previous studies on breath-holding (Hesser 1962, 1965) and on ventilatory responses to CO_2 (Fagraeus and Hesser 1970, Lambertsen et al 1973) in hyperbaric air, which indicate that high N_2 pressures (up to at least 6.0 ATA) have no significant narcotic influence upon neural structures involved in the control of respiration. Other studies have shown, on the other hand, that the ventilatory response to exercise (Tabakin and Hanson 1960, Cerretelli, Sikand and Farhi 1969) and CO_2 (Cherniack and Snidal 1956, Milic-Emili and Tyler 1963) are diminished when the work of breathing is increased at 1.0 ATA by addition of an external resistance. It may be concluded, therefore, that the non-O_2-dependent reduction of V_I observed at 4.5 ATA air was due mainly to increased airway resistance caused by the increased gas density.

The separate effect of high O_2 pressure on ventilation during maximal exercise ($V_E$max) was studied by comparing data obtained with He-O_2 at 3.0 ATA to those with air at 1.0 ATA (IV). The threefold increase in P_{O_2} in the presence of normal gas density in the He-O_2 experiments resulted in a significant reduction of $V_E$max (14 %), which is analogous to that observed with O_2-enriched gas mixtures at normal atmospheric pressure (cf. Asmussen and Nielsen 1946, 1955, Bannister and Cunningham 1954, Dcroanne et al 1973). The further 20 % reduction in $V_E$max observed when air was substituted for He-O_2 at 3.0 ATA provides a measure of the non-O_2-dependent decrease of $V_E$max during air breathing at this pressure (IV). Since the O_2-dependent decrease of $\dot{V}_E$max at normal atmospheric pressure has been found to be maximal already at an inspired O_2 concentration of 66 % (Bannister and Cunningham 1954), equivalent to the O_2 pressure in the present 3.0 ATA air experiments, it can be assumed that the O_2-induced reduction of $\dot{V}_E$max at 6.0 ATA was of similar magnitude as at 3.0 ATA. The 46 % reduction in $V_E$max observed at 6.0 ATA air would thus be predominantly due to non-O_2-dependent factors.

The importance of increased gas density in reducing the ventilatory capacity has been investigated by i.a. Maio and Farhi (1967), who found

gradual decrement in maximum voluntary ventilation with increased relative gas density no matter whether this was achieved by using a dense gas at normal atmospheric pressure by raising the ambient pressure or by a combination of both Increased gas density augments the airway resistance and thereby the work of breathing which has been suggested as a limiting factor for heavy exercise at depth (cf Lanphier 1967) Recent findings have shown however that the power dissipated by the ventilatory muscles during exhaustive exercise remains small as compared to the total work performed also when the ambient air pressure is raised to 3 0 ATA (Varène Jacquemin and L'Huillier 1972) An important consequence of increased gas density at high ventilatory rates is the flow limitation that occurs in the airways due to dynamic compression (for reviews see Lanphier 1969 Wood and Bryan 1971) It may therefore be surmised that the non-O_2-dependent reduction of $V_E$max observed at raised air pressures in the present experiments was largely due to such density induced flow limitation.

As a result of the reduced ventilatory responses to submaximal and maximal exercise in the present hyperbaric air experiments the CO_2 elimination was impeded with a consequent marked rise in end-tidal P_{CO_2} Thus the relative hyperventilation with regard to metabolically produced CO that is normally induced in exhaustive exercise (Asmussen 1965) was prevented at 3 0 and 6 0 ATA which must have resulted in a severe respiratory acidosis superimposed on the metabolic acidosis produced by lactate formation (II IV) Also the CO_2 tension in the blood is raised and the CO_2 transport from the tissues impeded by hyperoxia if the latter is great enough to cause an increase in the oxygen saturation of the venous blood (cf Lambertsen 1965)

Oxygen uptake

During submaximal exercise in air V_{O_2} at a given ergometric load was found to be about 10 % higher at 4 5 ATA than at 1 0 ATA (I) This rise in $\dot{V}_{O_2}$ can apparently not be ascribed to the increase in O_2 pressure since it has been shown that a similar rise in O_2 pressure during comparable work loads at normal ambient pressure exerts no significant influence on V_{O_2} (Asmussen and Nielsen 1955) Similarly it seems unlikely that the high N_2

pressure or the pressure per se would contribute to the increased $\dot{V}_{O_2}$ at 4 ATA as it has been shown that the oxygen consumption rat of isolat d t ssues such as the rat diaphragm or brain tissue remained unchang d when the N pressure was raised to 4 7 ATA at an O_2 pressure of 1 0 (St d) Riggs and Haugaard 1946 Rodgers Fenn and Craig 1969) It seems re s nable t con clude therefore that the higher than normal V_{O_2} at 4 0 ATA air wa caused by the increased work of breathing induced by the increase in gas dens ty and hence in airway resistance Further support for this conclusion an be obtained from studies by Glauser Glauser and Rusy (1967) and Salzano Ro h and Saltzman (1970) In experiments on the ventilatory response t C C n rmal atmospheric pressure Glauser et al found that the O_2 cost of br ath ng increas d significantly when the density of the inspired gas was incre sed by substituting SF_6 for nitrogen in the inhaled CO air mixture Salzano t al observed in exercise experiments that the V_{O_2} for a given ergometr l ad was significantly higher at 31 3 ATA breathing a mixture of helium-oxyg n the density of which was 4 4 times that of air at 1 0 ATA In both inv stiga tions the extra V_{O_2} found during inhalation of the denser gases was ascribed in large part or entirely to the increased work of breathing

During maximal exercise V_{O_2}max was significantly increased by 8 11 % at 1 4 ATA air (II III) This effect may be ascribed to the rise in O pressure since it is well stablished that V_{O_2}max increases significantly when extra oxygen is offered to the working muscles e g by breathing O_2-enriched gases at normal atmospheric pressure (Hill Long and Lupton 1924 Nielsen and Hansen 1937 Margaria et al 1961 197? Doroonn t l 1973) That the increased work of breathing due to increased gas density t 1 4 ATA air contributed to the rise in V_{O_2}max seems unlikely as it has be n shown that an increase in the external airway resistance and hence in wo k f breathing does not cause any significant increase of V_{O_2} max at 1 0 ATA air (Cerretelli Sikand and Farhi 1969 Demedts and Anthonisen 1973) When the ambient air pressure was raised to 2 0 and 3 0 ATA (II) no further increase in V_{O_2}max was observed in sp te of O_2 pressures which under no mal atmo spheric condit ons would yield clearcut increases in V_{O_2}max On the contrary

at 2 0 and 3 0 ATA air $\dot{V}_{O_2}$ max was lower than at 1 4 ATA and at 6 0 ATA air it was not significantly different from control The observation that with He–O_2 breathing at 3 0 ATA $\dot{V}_{O_2}$max increased by no less than 0 46 l min^{-1} whereas with air breathing at the same pressure no distinct change in $\dot{V}_{O_2}$max occurred (IV) indicates that factors related to the increased N_2 pressure opposed the beneficial influence of the high O_2 pressure on $\dot{V}_{O_2}$max. The clue to the unchanged $\dot{V}_{O_2}$max at 3 0 and 6 0 ATA air should be sought in the defective CO_2 elimination in hyperbaric air secondary to increased gas density and hypoventilation (cf above) For as shown in recent experiments at normal barometric pressure breathing through increased resistance (Cerretelli Sikand and Farhi 1969 Hermansen Vokac and Lereim 1972 Demedts and Anthonisen 1973) or the addition of 15 mm Hg P_{CO_2} to the inspired air (Luft Finkelstein and Elliott 1974) decreases the maximal O_2 uptake attainable during exercise A similar mechanism was presumably involved in the hyperbaric air experiments where the reduced ventilatory response to exercise led to marked hypercapnia. With the data at hand however it cannot be settled whether this "CO_2 effect acted by setting a limit due to respiratory distress, or by interfering with the supply or utilization of oxygen in the working muscles Also it cannot be excluded that N_2 molecules interfered with the diffusion of O_2 in the maximal exercise experiments in which metabolic rates were greatly increased

Heart rate

The results obtained during submaximal exercise in hyperbaric air (I, II V) confirm the old observation by Shilling Hawkins and Hansen (1936) that high pressures of air exert a depressant effect on heart rate an effect that these authors attributed to the elevated O_2 pressure The present data (I V) clearly demonstrate however that the relative bradycardia observed at 4 5 and 6 0 ATA air could only in part be explained by the high P_{O_2} the remaining non–O_2-dependent part being caused by the increased P_{N_2} gas density or ambient pressure per se acting either alone or in combination. It is well established that hyperoxia causes a decrease in heart rate at

submaximal work, both when induced at normal (Asmussen and Nielsen 195) and at high ambient pressures (Salzano et al 1967 Kaijser 1970 Taunton et al. 1970) This effect has been attributed to an increase in vagal tone since in the resting condition, it is prevented by administration of atropine (Daly and Bondurant 1962) Results from the present study (V) indicate however that during light exercise the major part of the O_2-dependent reduction of heart rate can be ascribed to a direct influence of hyperoxia on the heart whereas the remaining part is due to an increased parasympathetic tone

A non-O_2-dependent slowing of heart rate in submaximal exercise has recently been reported by Flynn Berghage and Coil (1972) to occur in humans breathing normoxic gas mixtures of varying density at constant or varying ambient pressures A similar effect has also been obtained during exercise in helium atmospheres at pressures up to and including 31 3 ATA (Hamilton 1967 Salzano Rausch and Saltzman 1970 Bradley et al 1971 Schaefer Carey and Dougherty 1971) Flynn et al. (1972) obtained evidence that this relative bradycardia was due to the combined effects of increments in ambient pressure and gas density although no explanation for the underlying mechanism was offered. In the isolated guinea-pig heart preparation Fagraeus (1971) on the other hand was unable to detect any effects of raising the N_2 pressure to 8 0 ATA or the hydrostatic pressure to 9 0 ATA on heart rate myocardial contractile force or the response to sympathetic stimulation. These results together with the findings in a recent study by Fagraeus Häggendal and Linnarsson (1973) that the mean arterial pressure and the noradrenaline levels in arterial blood during heavy exercise at 6 0 ATA air showed no consistent changes in the face of a lowered heart rate seem to indicate that the non-O -dependent reduction in heart rate is due to a subnormal response to sympathetic stimulation. This concept is supported by the present observation that the non-O_2-dependent slowing of the heart was significantly less after beta adrenergic blockade (V)

Data about heart rate during maximal exercise in hyperbaric air are lacking in the literature In the present investigation the reduction in heart

rate was smaller during maximal than during submaximal exercise at air pressures up to and including 3 0 ATA (II) A possible explanation for this discrepancy in the heart rate responses may be that HR_{max} is not influenced by O_2 pressures up to 1 0 ATA (cf Miller et al. 195_ Margaria et al 197_ Dorcanne et al. 1973) Thus the observed decreases in HR_{max} in the present investigation were apparently of non-O_2-dependent origin (II IV) The observation of a reduced HR_{max} in the He-O_2 experiments at 3 0 ATA (IV) indicates an effect on the heart either by the helium gas itself or by the pressure per se The further reduction in HR_{max} induced by substituting air for He-O_2 at 3 0 ATA (IV) was apparently caused either by the increased gas density or the raised N_2 pressure The additional reduction of HR_{max} in the 6 0 ATA air condition (IV) may be similarly explained as caused by the increase in gas density N_2 pressure or pressure per se or by a combination of these factors Although the true nature of this non-O_2-dependent reduction of HR_{max} is not known it seems likely that it can be attributed to a subnormal response of the heart to adrenergic stimulation in conformity with the situation in submaximal exercise discussed above (cf V Fagraeus Häggendal and Linnarsson 1973) Since the cardiac stroke volume during maximal exercise has been shown to be unaffected both by an increase of the inspired O_2 pressure to 0 5 ATA (Ekblom 1974) or 3 0 ATA (Kaijser 1970) and by beta receptor blockade (Epstein et al 1965) it seems probable that the changes of HR_{max} in the present study do reflect corresponding changes of cardiac output i e reductions of cardiac output of only about 2 % in the He-O_2 experiments at 3 0 ATA and 4 5 % in the air experiments at 3 0 and 6 0 ATA (IV)

Point of exhaustion

It has been the objective of many studies to find a causal connection between a biochemical or a physiological variable on the one hand and the subjective perception of exhaustion on the other A major problem is that several factors contribute to setting the individual perception of the point of exhaustion, depending i.a on the type of maximal exercise test that is performed Psychological factors such as motivation familiarity with the

different experimental conditions and tolerance to discomfort and pain experienced in exhaustive exercise are important In the present investigation all subjects were well acquainted with both the maximal exercise procedure and with exposure to raised ambient air pressures Thus any influence of psychological factors on the point of exhaustion was minimized except possibly in the 6 0 ATA air experiments where respiratory distress and dizziness were present in varying degrees Among physiological factors a depletion of the glycogen store in the muscle cell seems unlikely as a limiting factor since all maximal exercise tests were of relatively short duration (cf Saltin and Karlsson 1971) Increased acidity in the muscle cell is known to decrease enzyme activity (cf. Danforth 1965) and a decreased pH in the muscle (Kaijser 1970 Hermansen and Osnes 1972) or in the blood (Cerretelli 1967) has been proposed as a limiting factor for exhaustive exercise of short duration. Accumulation of lactate with a consequent increased acidity in the muscle cell has recently been suggested by Hultman and Bergström (1973) to be a major factor in bringing about muscular fatigue either through inhibition of glycolysis or by preventing the rate of glycolysis to increase during the terminal part of exercise

In the present investigation peak blood lactate and muscle lactate at exhaustion remained unchanged no matter whether maximal exercise was performed in normoxia or hyperoxia or whether hypocapnia or marked hypercapnia was present (II, III IV) This observation strongly favors the concept that maximal work performance is more closely related to the lactate concentration than to the P_{CO_2} or pH levels in the blood or in the working muscles at the point of exhaustion. It would thus seem that maximal work is limited by a critical level of lactate concentration possibly by inducing a product inhibition of lactic dehydrogenase (LDH) a hypothesis recently suggested by Karlsson, Hultén and Sjödin (1974) based on an in vitro study of human skeletal muscle LDH.

The shorter endurance time in the 3 0 and 6 0 ATA air experiments compared to that observed at 3 0 ATA He–O_2 would therefore indicate that

the rate of lactate accumulation was higher in the former conditions although the inspired O pressure was the same or higher The explanation for this increase in the rate of lactate accumulation may be sought in the same mechanisms as were responsible for the failure of V_{O_2}max to increase in the 3 0 and 6 0 ATA air experiments (cf above)

GENERAL SUMMARY

The bulk of physiological research on muscular exercise has so far been restricted mainly to the normal atmospheric environment. To investigate the effects of acute exposure to the hyperbaric environment on physiological functions in submaximal and maximal exercise, 21 healthy subjects were exposed to increased ambient pressures in the range 1.0 - 6.0 ATA. The main objective of this investigation has been to evaluate the separate and combined effects of high O_2 and N_2 pressures on cardiorespiratory and metabolic functions. This was done by comparing exercise data obtained under different ambient conditions, i.e. with the subjects breathing air, O_2, or He-O at various elevated pressures (cf. Table I, p. 11).

A. Submaximal work

1. Oxygen uptake and carbon dioxide elimination increased linearly with the work load both at 1.0 and 4.5 ATA air. Both variables showed a tendency to be higher in the hyperbaric condition and at 150 W V_{O_2} was increased by 12 % above control. There were no gross differenc s between the increases in V_{O_2} and V_{CO_2} as reflected by an unchanged respiratory exchange ratio when the air pressure was raised to 4.5 ATA.

2. Whereas pulmonary ventilation at 150 W decreased significantly by 9 % when the O_2 pressure was increased by substituting O_2 for air at 1.0 ATA, no reduction of ventilation was observed at 4.5 ATA air as compared t control in spite of the raised O pressure (1.0 ATA). Since V_{O_2} was increased above control at 4.5 ATA air it follows, howe er, that ent lat on at a given V_{O_2} was lower in the hyperbaric condit on. At a V_O of 2.0 l. min $^{-1}$ STPD, the total v ntilatory reduction at 4.5 ATA amounted t approximately 17 % as compared to 1.0 ATA air, about half of which could be ascribed to the O_2-dependent decrease in ventilation. The remaining part

1 0 the non-O_2-dependent reduction of ventilation could be attributed mainly to the higher gas density causing an increase in airway resistance and hence in the work of breathing

3 As a consequence of the reduction in ventilation at 4 5 ATA air end-tidal P_{CO_2} was significantly raised at all three work levels (50 100 and 150 W) During 150 W at 4 5 ATA end-tidal P_{CO_2} was 18 % higher than control whereas the corresponding value at 1 0 ATA O_2 was 7 % above control reflecting the O_2-dependent decrease in ventilation.

4 Heart rate was significantly decreased at all work levels during exposure to 4 5 ATA the decreases being more evident when related to V_{O_2} The subnormal heart rate response with hyperbaric air could only in part be ascribed to the raised O_2 pressure the remaining part being caused by some factor related to the increase in N_2 pressure By the use of differentiated autonomic blockade evidence was obtained that the O_2-dependent decrease in heart rate was due both to a direct effect of O_2 on the heart and to an increased para sympathetic tone whereas the non-O_2-dependent reduction in heart rate *seems to be caused by a change in the beta-adrenergic stimulation of the heart*

B. Maximal work

5 Maximal oxygen uptake was significantly increased by 8-11 % at 1 4 ATA air the increase being attributable to the rise in O_2 pressure At ambient pressures in excess of 1 4 ATA however no further increase in V_{O_2}max was observed in spite of the additional rise in O_2 pressure Instead V_{O_2}max at 2 0 and 3 0 ATA air was lower than at 1 4 ATA and at 6 0 ATA air was not significantly different from control at 1 0 ATA air The observation that V_{O_2}max was significantly increased by 13 % at 3 0 ATA He-O_2 indicates that factors related to the increased N_2 pressure were responsible for the observed failure of V_{O_2}max to increase when the air pressure was raised beyond 1 4 ATA

6 The maximal exercise ventilation decreased progressively as the ambient air pressure was raised At 1 4 ATA the decrease averaged 7 %, at 2 0 ATA

18 % and at 6 0 ATA 46 %. The observation of a 14 % reduction in pulmonary ventilation at 3 0 ATA He-O_2 reflected the O_2-dependent part of the 33 % reduction in ventilation observed during air breathing at the same pressure

7 The inadequate ventilatory response to maximal exercise at raised air pressures resulted in a progressive rise in end-tidal P_{CO_2} at the point of exhaustion. With He-O_2 at 3 0 ATA only a slight increase in end-tidal P_{CO_2} was observed corresponding to the O_2-dependent reduction in ventilation.

8 Maximal heart rate remained unchanged as the air pressure was raised to 1.4 ATA but then decreased at higher pressures At 3 0 and 6 0 ATA air HR_{max} was significantly lower than control During He-O breathing at 3 0 ATA HR_{max} was significantly lower than control but consistently higher than during air breathing at the same pressure

9 Muscle metabolites (ATP CP lactate pyruvate glucose glucose-6-phosphate) glycogen depletion and oxygen deficit at the point of exhaustion remained largely unchanged when the air pressure was raised to 1 4 ATA despite the significant increase in $V_{O_2}max$.

10 Peak blood lactate did not differ from control in any condition studied whereas endurance time was related to the ambient pressure in a manner similar to $V_{O_2}max$. This observation, together with the finding of a marked hypercapnia at 3 0 and 6 0 ATA air suggests that maximal work performance was more closely related to lactate concentration than to the P_{CO_2} or pH levels existing in the blood or in the working muscles at the point of exhaustion.

REFERENCES

ASMUSSEN E. Muscular exercise In. Handbook of Physiology Respiration edited by W O Fenn and H Rahn. Washington D C Am Physiol Soc 1965 Sect 3 Vol II Chapt. 36 939 978

ASMUSSEN E and M. NIELSEN Studies on the regulation of respiration in heavy work. Acta physiol scand 1946 12 171-188

ASMUSSEN E and M. NIELSEN The cardiac output in rest and work at low and high oxygen pressures Acta physiol scand 1955 35 73-83

ASMUSSEN E and M NIELSEN Physiological dead space and alveolar gas pressures at rest and during muscular exercise Acta physiol scand 1956 38 1-21

ASMUSSEN E and M NIELSEN Pulmonary ventilation and effect of oxygen breathing in heavy exercise Acta physiol scand 1958. 43 365-378

ÅSTRAND IRMA Aerobic work capacity in men and women with special reference to age Acta physiol scand 1960 49 Suppl 169 1 92

ÅSTRÖM T and O WIGERTZ A digital computer for automatic breath-by--breath calculation of respiratory functions Report Lab Aviat. Naval Med Karol Inst Stockholm March 1966 23 pp.

BANNISTER R. G and D J C CUNNINGHAM The effects on the respiration and performance during exercise of adding oxygen to the inspired air J Physiol (Lond) 1954 125 118-137

BARKER S. B. and W H SUMMERSON The colorimetric determination of lactic acid in biological material J biol Chem 1941 138 535-554

BENNETT P B. Inert gas narcosis In: The Physiology and Medicine of Diving edited by P B. Bennett and D H Elliott. London. Baillière Tindall and Cassel. 1969 155-182

BERGSTRÖM J Muscle electrolytes in man. Scand J clin. Lab Invest 1962 14 Suppl 68 1-110

BERT P La Pression Barométrique Recherches de Physiologie Expérimentale Paris Masson. 1878 1168 pp

BOYCOTT A E G C C DAMANT and J S. HALDANE The prevention of compressed air illness J Hyg (Lond) 1908 8 342-443

BRADLEY M E N R. ANTHONISEN J VOROSMARTI and P G LINAWEAVER Respiratory and cardiac responses to exercise in subjects breathing helium-oxygen mixtures at pressures from sea level to 19 2 atmospheres In Proc Fourth Symposium on Underwater Physiology edited by C J Lambertsen. New York and London. Academic Press 1971 325-337

BRISMAR J C M. HESSER and G MATELL Breath-by breath sampling of alveolar (end-tidal) gas Acta physiol scand 1962 56 299-305

BROUSSOLLE B. E BENSIMON A MICHAUD et C VEGEZZI Comparison des réponses ventilatoires et des pressions partielles alvéolaires de CO_2 de plongeurs sous marines entraînés et de témoins non plongeurs au cours du travail musculaire en atmosphère hyperbare In. Proc, Third International Conference on Hyperbaric and Underwater Physiology Paris Doin. 1972 80-87

CERRETELLI P Lactacid O_2 debt in acute and chronic hypoxia In. Exercise at Altitude edited by R. Margaria. Amsterdam Excerpta Medica Foundation. 1967 58-64

CERRETELLI P R S. SIKAND and L. E FARHI Effect of increased airway resistance on ventilation and gas exchange during exercise J appl Physiol 1969 27 597 600

CHERNIACK R. M. and D P SNIDAL The effect of obstruction to breathing on the ventilatory response to CO_2 J clin. Invest. 1956 35 1 86 1290

COOK, J C Work capacity in hyperbaric environments without hyperoxia. Aerospace Med 1970 41 1133 1136

DALY W J and S. BONDURANT Effects of oxygen breathing on the heart rate blood pressure and cardiac index of normal men -- resting with reactive hyperemia and after atropine J clin. Invest 1962 41 126-132

DANFORTH W H. Activation of glycolytic pathway in muscle In Control of Energy Metabolism edited by B. Chance R. W Estabrook and J R. Williamson. New York and London. Academic Press 1965 287 297

DEMEDTS M. and N R. ANTHONISEN Effects of increased external airway resistance during steady state exercise J appl. Physiol 1973 35 361 366

DEROANNE R. J DUJARDIN M LAMY R. MARECHAL J M. PETIT and F PIRNAY Effect of hyperbaric oxygenation on maximum aerobic work capacity Försvarsmedicin (Stockholm) 1973 9 352 356

DÖBELN W von A respiratory valve with insignificant dead space Acta physiol scand 1949 18 34 35

EAGAN C J and E R. PLESE Cardiorespiratory and metabolic effects of work during hypo- and hyperbaria Fed Proc 1969 28 p 593

EKBLOM B Personal communication. 1974

EPSTEIN S E B. F ROBINSON R. L. KAHLER and E BRAUNWALD Effects of beta adrenergic blockade on the cardiac response to maximal and submaximal exercise in man. J clin. Invest 1965 44 1745-1753

FAGRAEUS L. Performance of the isolated guinea-pig heart in hyperbaric environment Report Lab Aviat Naval Med Karol Inst. Stockholm July 1971 11 pp.

FAGRAEUS L. and C M HESSER Ventilatory response to CO_2 in hyperbaric environments Acta physiol. scand. 1970 80 19A-20A.

FAGRAEUS L. J HÄGGENDAL and D LINNARSSON Heart rate arterial blood pressure and noradrenaline levels during exercise with hyperbaric oxygen and nitrogen. Försvarsmedicin (Stockholm) 1973 9 265-270

FENN W O The physiological effects of hydrostatic pressures In. The Physiology and Medicine of Diving edited by P B. Bennett and D H Elliott London Baillière Tindall and Cassel. 1969 36-57

FISHER R. A. Statistical Method for Research Workers 10th Ed. Edinburgh Oliver and Boyd Ltd 1948 354 pp.

FLYNN E T T E. BERGHAGE and E F COIL Influence of increased ambient pressure and gas density on cardiac rate in man. Experimental Diving Unit Report 4-72 Washington Navy Yard Washington D C 1972 35 pp.

GLAUSER S. C. E M. GLAUSER and B. F RUSY Gas density and the work of breathing Respir Physiol 1967 2 344-350

GLOSSARY COMMITTEE of the IUPS Glossary on respiration and gas exchange J appl Physiol 1973a 34 549-558

GLOSSARY COMMITTEE of the IUPS Glossary of terms for thermal physiology J appl. Physiol 1973b 35 941-961

HAMILTON R. W Jr Physiological responses at rest and in exercise during saturation at 20 atmospheres of $He-O_2$ In. Proc. Third Symposium on Underwater Physiology edited by C. J Lambertsen. Baltimore The Williams & Wilkins Co 1967 361 374

HERMANSEN L. and J -B. OSNES Blood and muscle pH after maximal exercise in man. J appl. Physiol. 1972 32 304 308

HERMANSEN L. Z VOKAC and P LEREIM Respiratory and circulatory response to added air flow resistance during exercise Ergonomics 1972 15 15-24

HESSER C M. The role of nitrogen in breath holding at increased pressures In. Man s Dependence on the Earthly Atmosphere edited by K. E Schaefer New York: Macmillan. 1962 327 334.

HESSER C M. Breath holding under high pressure In. *Physiology of Breath Hold Diving and the Ama of Japan* edited by H Rahn and T Yokoyama. Natl Acad. Sci – Natl. Res Council. Publ 1341 1965 165–181

HILL A V C N H LONG and H LUPTON Muscular exercise lactic acid and the supply and utilisation of oxygen. Proc roy Soc B. 1924 97 Part VII. 155–167

HOFF E. C A Bibliographical Sourcebook of Compressed Air Diving and Submarine Medicine. Washington D C Navy Dept. Bur Med Surg 1948 382 pp

HULTMAN E and J BERGSTRÖM Local energy supplying substrates as limiting factors in different types of leg muscle work in normal man. In. Limiting Factors of Physical Performance edited by J Keul. Stuttgart. Georg Thieme Publishers 1973 113 125

JARRETT A S. Alveolar carbon dioxide tension at increased ambient pressures J appl Physiol. 1966 21 158–162.

KAIJSER L. Limiting factors for aerobic muscle performance Acta physiol scand 1970 79 Suppl. 346 1–96.

KARLSSON J Lactate and phosphagen concentrations in working muscle of man. Acta physiol scand 1971. 81 Suppl. 358 1–72

KARLSSON J BODIL HULTEN and B SJÖDIN Substrate activation and product inhibition of LDH in human skeletal muscle Acta physiol scand. In press

LALLY D A. T O. MOORE and S. K. HONG Cardio-respiratory responses to exercise in air and water at 1 and 2 ATA. Report Dept. of Physiol Univ. of Hawaii School of Medicine Dec 1971 25 pp

LAMBERTSEN C J Effects of oxygen at high partial pressure In Handbook of Physiology Respiration, edited by W O. Fenn and H Rahn Washington D C Am Physiol Soc 1965 Sect 3 Vol II. Chapt. 39 1027 1046

LAMBERTSEN C J. S. G OWEN H. WENDEL M. W STROUD A A. LURIE W LOCHNER and G F CLARK, Respiratory and cerebral circulatory control during exercise at 21 and 2 0 atmospheres inspired pO_2 J appl. Phys ol. 1959 14 966–982

LAMBERTSEN C J R GELFAND M. J LEVER G BODAMMER N TAKANO T A. REED J G DICKSON and P T WATSON Respiration and gas exchange during a 14-day continuous exposure to 5 2 ℅ O [illegible] pressure equivalent to 100 FSW (4 ata) Aerospace Med 1973 44

LANPHIER E H Influence of increased ambient pressure upon alveolar ventilation. In Proc Second Symposium on Underwater Physiology edited by C J Lambertsen and L J Greenbaum Jr Washington D C Natl. Acad Sci — Natl Res Council Publ 1181 1963 124 133

LANPHIER, E H Interactions of factors limiting performance at high pressures. In Proc Third Symposium on Underwater Physiology edited by C J Lambertsen. Baltimore The Williams & Wilkins Co 1967 375-385

LANPHIER E H Pulmonary function. In. The Physiology and Medicine of Diving edited by P B. Bennett and D H Elliott. London: Baillière Tindall and Cassel 1969 58-11.

LINDBORG B. O WIGERTZ and Th ÖDMAN A beat-to-beat heart rate meter with linear analog output for muscular exercise studies Report Lab Aviat. Naval Med Karol Inst Stockholm Dec. 1969 23 pp.

LUFT U C. S. FINKELSTEIN and J C ELLIOTT Respiratory gas exchange acid base balance and electrolytes during and after maximal work breathing 15 mm Hg P_{ICO_2} In. Proc of a Satellite Symposium of the XXV International Congress of Physiological Sciences 1971 on CO_2 and Metabolic Regulations. New York: Springer In press

MAIO D A. and L E FARHI Effect of gas density on mechanics of breathing J appl. Physiol. 1967 23 687 693

MARGARIA R. E CAMPORESI P AGHEMO and G SASSI The effect of O_2 breathing on maximal aerobic power Pflüg Arch. ges Physiol 1972 336 225-235

MARGARIA R. P CERRETELLI S. MARCHI and L. ROSSI Maximum exercise in oxygen. Int Z angew Physiol. 1961 18 465-467

MILIC-EMILI J and J M. TYLER Relation between work output of respiratory muscles and end-tidal CO_2 tension. J appl Physiol 1963 18 497 504

MILLER A T Jr H L. PERDUE E L. TEAGUE Jr and J S. FEREBEE Influence of oxygen administration on cardiovascular function during exercise and recovery J appl. Physiol. 1952 5 165-168

MILLER J N O D WANGENSTEEN and E H LANPHIER Ventilatory limitations on exertion at depth. In Proc. Fourth Symposium on Underwater Physiology edited by C J Lambertsen. New York and London. Academic Press 1971 317-323

MILLER J N O D WANGENSTEEN and E H LANPHIER Respiratory limitations to work at depth In. Proc Third International Conference on Hyperbaric and Underwater Physiology Paris Doin. 1972 118-123

NIELSEN M. und O HANSEN Maximale körperliche Arbeit bei Atmung O_2-reicher Luft Skand Arch. Physiol. 1937 76 37-59

RODGERS S.H W O FENN and A B. CRAIG Jr The oxygen consumption of rat tissues in the presence of nitrogen helium or hydrogen. Respir Physiol. 1969 6 168-177

SALTIN B. and J KARLSSON Muscle glycogen utilization during work of different intensities In. Muscle Metabolism during Exercise edited by B. Pernow and B. Saltin. New York and London Plenum Press 1971 289-299

SALZANO J D C. RAUSCH and H A SALTZMAN Cardiorespiratory responses to exercise at a simulated seawater depth of 1 000 feet. J appl. Physiol. 1970 28 34-41

SALZANO J W H BELL W B. WEGLICKI and H A. SALTZMAN Metabolic respiratory and hemodynamic responses to exercise at increased oxygen pressure In. Proc Third Symposium on Underwater Physiology, edited by C J Lambertsen. Baltimore The Williams & Wilkins Co. 1967 351 360

SCHAEFER K.E C.R. CAREY and J H DOUGHERTY Jr Pulmonary function and respiratory gas exchange during saturation-excursion diving to pressures equivalent to 1000 feet of seawater In Proc Fourth Symposium on Underwater Physiology edited by C J Lambertsen. New York and London. Academic Press 1971 35 370

SCHOLANDER P F Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. J biol Chem 1947 167 235-250

SCHOLTZ R. H. SCHMITZ T BUECHER and J O LAMPEN Über die Wirkung von Nystatin auf Bäckerhefe Biochem Z 1959 331 1 86

SHILLING C W J A. HAWKINS and R.A. HANSEN The influence of increased barometric pressure on the pulse rate and arterial blood pressure U S. Navy Med. Bull. 1936 34 39-47

STADIE W C. B.C RIGGS and N HAUGAARD Oxygen poisoning III. The effect of high oxygen pressures upon the metabolism of brain. J biol Chem. 1945 160 191 98

STRÖM G The influence of anoxia on lactate utilization in man after prolonged muscular work. Acta physiol. scand 1949 17 440-451

TABAKIN B.S. and J S. HANSON Response to ventilatory obstruction during steady-state exercise J ppl. Physiol 1960 15 579-582

TAUNTON J E E W BANISTER, T R. PATRICK P OFORSAGD and W R. DUNCAN Physical work capacity in hyperbaric environments and conditions of hyperoxia. J. appl Physi l 1970 28 421-4 7

VARENE P Ch. JACQUEMIN et J L'HUILLIER Les performances ventilatoires en plongée In Proc Third Int rnational Conf rence on Hyperbari and Und rwater Physiology Paris Doin. 1972 85-94

WIGERTZ O A low resistance flow meter for wide range ventilatory measurement. Respir Physiol. 1969 7 263 270

WOOD L.D H. and A.C BRYAN Mechanical limitations of exercise ventilation at increased ambient pressure In. Proc Fourth Symposium on Underwater Physiology edited by C J Lambertsen. New York and London. Academic Press 1971 307 316

WYNDHAM C H N B. STRYDOM A.J van RENSBURG and G G ROGERS Effects on maximal oxygen intake of acute changes in altitude in a deep mine J appl Physiol. 1970 29 552 555

Snabbtryckeriet AB Stockholm 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 415

Dynamics of Pulmonary Gas Exchange and Heart Rate Changes at Start and End of Exercise

by

Dag Linnarsson

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 415

From the Departments of Aviation and Naval Medicine
Faculty of Medicine Karolinska Institutet
Stockholm Sweden

Dynamics of Pulmonary Gas Exchange and Heart Rate Changes at Start and End of Exercise

by

Dag Linnarsson

STOCKHOLM 1974

ISBN 91–7222–082–1
Bundt Offset Stockholm 74

CONTENTS

I	PREFACE	5
	Symbols and Abbreviations	6
II.	INTRODUCTION	7
III	METHODS	10
	Subjects and Experimental Procedure	10
	Techniques Equipment	13
	Analog On-Line Computations	15
	Digital Off Line Computations	17
IV	RESULTS	24
	Maximal Work Tests	24
	Responses to Step Changes in Submaximal Work Load	24
V	DISCUSSION	42
	A Methodological Considerations	42
	Maximal O_2 Uptake	42
	Breath-by Breath Measurements during Submaximal Work	42
	Experimental Conditions	44
	Parameterization and Data Reduction	46
	B General Discussion	47
	Dynamic Behavior of $\dot{V}_E$ V_T and FRC in Response to Step Changes in Work Load	47
	Dynamic Behavior of Heart Rate in Response to Step Changes in Work Load	49
	Interrelation Between Ventilatory and Heart Rate Dynamics	52
	Dynamic Behavior of V_{O_2} in Response to Step Changes in Work Load	54
	Dynamic Behavior of V_{CO_2} and R in Response to Step Changes in Work Load	61
VI	GENERAL SUMMARY	62
VII	REFERENCES	65

I PREFACE

The present investigation is part of a research project dealing with the influences of various stress factors on the pulmonary gas exchange and circulatory functions. The experimental part of the investigation was carried out during the years 1972–1973

I wish to express my profound thanks to Professor Hilding Bjurstedt, Head of the Department of Aviation Medicine and to Professor Carl Magnus Hesser Head of the Department of Naval Medicine for their generous support and active interest through all phases of this work

I am also indebted to Dr Gunnar Rosenhamer who originally encouraged me to work in the area of environmental physiology to Professor Ove Wigertz for having introduced me into the use of systems analysis in physiology to Dr Arne Granath for performing the pulmonary function tests in his laboratory and to Dr Lennart Fagraeus, Professor Bengt Saltin and Dr Jan Karlsson for stimulating advice and fruitful discussions. For never failing assistance throughout this study I am most indebted to my technical colleagues Hans Karlsson and Bertil Lindborg

I am also especially grateful to Barbro Bergström, Karin Vasser and Bengt Macarz for their skilful assistance in the processing of data Gunilla Haggård and Irène Unander Scharin for capable secretarial work, and to Carl Huldt Berndt Lennholm, Bertil Lundin and Anders Truvé for excellent technical assistance

The work has been supported in full by grants from the Swedish Medical Research Council (projects 40X–680 and 40X–682)

Stockholm May 1974

Dag Linnarsson

Symbols and Abbreviations

Primary Symbols

V	Gas volume in general
V	Gas volume per unit of time
P	Pressure in general including partial pressure
F	*Fractional concentration in gas phase*

Suffixes

I	Inspired gas
A	Alveolar gas
E	Expired gas
T	Tidal gas
ET	End-tidal gas
a	Arterial blood
$\bar{v}$	Mixed venous blood

Some other Symbols and Abbreviations

STPD	Standard temperature and pressure dry (0^{o}C 760 mm Hg)
BTPS	Body temperature and pressure saturated with water vapor
BTPD	Body temperature and pressure dry
FRC	Functional residual capacity
HR	Heart rate
$\dot{V}_{O_2(PC)}$	Rate of oxygen transfer between the alveolar space and the pulmonary capillaries volume per unit of time
V_L	Lung volume
WL	Work Load
AWL	Absolute work load
RWL	Relative work load
T_D	Time delay
a	Amplitude
τ	Time constant
MRT	Mean response time
l	Liter
mM	Millimoles per liter
min	Minute
sec	Second
msec	Millisecond
Hz	Cycles per second
W	Watt (1 W = 6 1 kpm/min)

Other symbols are defined in the text as they occur

II INTRODUCTION

The ultimate purpose of ventilation and circulation is to provide oxygen for the various energy consuming processes in the body and to remove the carbon dioxide formed during the same processes During muscular exercise the O_2 need can be increased more than tenfold compared to resting conditions Despite such large changes in metabolic rate there is a remarkable constancy of the internal environment in terms of for example arterial blood gas concentrations during steady-state conditions It is therefore generally agreed that the various respiratory and circulatory adjustments that accompany different intensities of physical activity are closely geared to the metabolic rate Recent reviews concerning respiratory circulatory and metabolic adjustments to muscular exercise include those of Asmussen (1965) Bevegård and Shepherd (1967) Edwards (1969) and Rowell (1974) Reviews on related topics may also be found in the proceedings of two recent symposia on "Physiology of Muscular Exercise" (edited by Chapman 1967) and "Muscle Metabolism during Exercise" (edited by Pernow and Saltin 1971).

Knowledge about the steady states of various physiological adjustments is generally insufficient in attempts to explain the regulatory mechanisms underlying the adaptational changes. Additional useful information may be found in the time patterns of the various changes involved and such information is increasingly used to study problems of fundamental importance in physiology Much of our present knowledge about the physiology of exercise is derived from studies of the changes occurring during the unsteady states fol lowing onset of work or during recovery Since the increased uptake of O_2 during exercise reflects the integrated action of a multitude of respiratory circulatory and metabolic changes, measurements of changes in O_2 uptake particularly in terms of O_2 deficit and O_2 debt have been found to be useful tools in studies on exercise physiology In 1913 Krogh and Lindhard used the changes in O_2 uptake during the initial stage of exercise to study the changes in pulmonary blood flow during exercise The work of Margaria Ed wards and Dill (1933), introducing the concept of the alactacid and the lactacid portions of the O_2 debt provides another example of an important contribution in exercise physiology based on measurements of O_2 uptake in the unsteady state The analysis and presentation of experimental data in the study of Margaria *et al.* were greatly facilitated by the use of a mathematical model for the time course of O_2 uptake

Traditionally interest in the time course of O_2 uptake has been focused primarily on the recovery period and the corresponding events at the onset of work have not been studied to the same extent. Berg (1947) published recordings of the response of O_2 uptake during onset of work and recovery but analyzed only the recovery responses.

by De Moor (1954) and Henry and De Moor (1956) showed that the increase of O_2 uptake in early exercise followed an exponential time course and Margaria *et al.* (1965) described this time course as a monoexponential function with a half time of about 30 sec In view of the diversity of processes that are involved in the response to exercise such a monoexponential model appears surprisingly simple It may be assumed that the results of earlier investigations were influenced by the lack of facilities for continuous measurements of O_2 uptake It is therefore interesting that with the advent of new research tools such as fast response gas analyzers and computer facilities, breath-by-breath measurements of the pulmonary-capillary O_2 transfer made it possible for Auchincloss Gilbert and Baule (1966) to confirm the observation by Krogh and Lindhard (1913) that O_2 uptake showed a sharp initial rise at the onset of exercise Henry and De Moor (1956) and later Whipp and Wasserman (1972) using a breath-by-breath technique showed that the steady state O_2 uptake was appreciably delayed with heavy work The notion that the slowing of the change in O_2 uptake depends on the relative rather than the absolute level of work finds support in the results of Linnarsson *et al.* (1974) who observed that when the aerobic work capacity was decreased with hypoxia the O_2 deficit for a given submaximal work load became increased Correspondingly the increased aerobic work capacity during hyperoxia was found to be accompanied by a decreased O_2 deficit at the same submaximal work load. On the other hand Åstrand and Saltin (1961) and Margaria *et al.* (1965) found that the maximal level of O_2 uptake was attained more rapidly the higher the external work load With the use of various techniques and experimental conditions, the time course of O_2 uptake has thus been described as being either unchanged slowed or accelerated with increasing work load However no systematic study seems to have been performed relating the directly measured time courses of O_2 uptake to the absolute and relative work intensity and to other cardiorespiratory functions that are known to be influenced by changes in work intensity

In the study of the dynamic behavior of physiological functions the introduction of certain concepts used in control engineering has proven useful for example in studies on respiratory regulation (Grodins *et al.* 1954 Milhorn *et al.* 1965 Wigertz 1971 Reynolds and Milhorn 1973 Fujihara, Hildebrandt and Hildebrandt 1973a, b Gelfand and Lambertsen 1973) A common approach has been to regard the physiological processes under study as a "black box" *i.e.* a system with unknown properties Well-defined changes, *e.g* in alveolar P_{CO_2} or work load are then used as input functions to the "black box" The time course of some dependent variable *e.g* the pulmonary ventilation provides the output (response) of the system under study By comparing the time patterns of the input and output functions, certain properties of the interposed system may be studied On the basis of the relationship of output and input functions, mathematical models can often be used to describe the dynamic behavior of the system under study

The limitation of the "black box" approach is that it can only describe what a system does, but not how it does it The usefulness of this approach is primarily that hypotheses that are consistent with the characteristics of the system can be distinguished from hypotheses which are not (*Fujihara et al 1973a*) *Recalling the study of Margaria et al (1933)* their approach was in essence that of a systems analysis, although the terminology of control engineering was not used Thus a step change in work load was used as the input function and the relationship between the time courses of the output and input functions was studied The dynamic properties of the unknown system were described in terms of a mathematical model which was found to be consistent with the hypothesis that there are two compartments of the O_2 debt

The purpose of the present investigation was to further analyze the time courses of respiratory gas exchange during the unsteady states of muscular exercise using recent advances in breath-by-breath recording technique and a systems analysis approach to quantify the time patterns during various conditions More specifically the objectives of this work may be summarized as follows

1 To further develop methods for the continuous breath-by-breath measurement of O_2 uptake and pulmonary capillary O_2 transfer during unsteady states of exercise and to study the time courses of these variables together with those of ventilation and heart rate by the use of analog and digital computation

2 To identify and quantify the various time components of the pulmonary gas exchange and heart-rate responses during step changes in ergometric work load using a systems *analysis approach*

3 To relate changes in the dynamic behavior of the measured variables to the work level in such a way that influences of the absolute work intensity could be distinguished from those of the relative work intensity

4 To relate the various time components of the $\dot{V}_{O_2}$ response to submaximal exercise to simultaneously measured ventilatory and heart rate responses

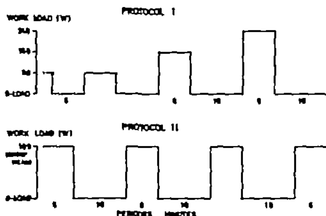


Fig. 1 Experimental protocols presented as work-load time profiles. Each of the protocols includes 3 positive and 3 negative step changes in work load. Each subject performed Protocol I on 3 occasions, breathing air and Protocol II with three different inspired gases: 14 21 and 30 % O_2 in N_2 The dotted lines denote warm-up periods.

separated by 10 min 0-load periods the first 6 min of work being a warm-up period For each subject a work load demanding approximately 65 per cent of $V_{O_2}max$ during air breathing was selected Each subject performed the Protocol II experiments once during air breathing (average $P_{I_{O_2}}$ = 148 mm Hg) once during inhalation of about 14 per cent O_2 in N_2 (average $P_{I_{O_2}}$ = 99 mm Hg) and once during inhalation of about 30 per cent O_2 in N_2 (average $P_{I_{O_2}}$ = 214 mm Hg) Thus from each of these experiments average responses to the same absolute work load could be obtained and then compared between normoxia hypoxia and hyperoxia. Immediately following the last work period in the Protocol II experiments a period of maximal work was performed using a work load known to lead to exhaustion within 4–5 min during air breathing. $V_{O_2}max$ was deter mined as above and post-exercise peak *blood lactate concentrations* were obtained from arterialized capillary samples from the fingertip and analyzed according to Barker and Summerson (1941) as modified by Ström (1949)

The subjects performed the experiments in a randomized order and they were not informed in advance about the exact nature of the exercise protocol or the composition of the inhaled gas. All experiments were started by 10 min of rest, with the subject breathing the preselected gas from the respiratory circuit The following 6-min period of "warm-up" exercise completed the equilibration period. Throughout the experiments care was taken to ensure an environment free from disturbing noise or other extraneous influences.

Techniques Equipment

The general experimental arrangement is shown in Fig 2 The subject was seated on an electrically braked *cycle ergometer* (Model AM 368 Elema, Sweden). The current through the load resistors was monitored and recorded to indicate the time and the nature of the work load (WL) changes The work load could be changed without the subjects prior knowledge at exact preset time intervals using an electronic time relay *Respiratory circuit* In order to avoid dryness of the upper airways when breathing continuously in a mouthpiece during intervals of 1 hour or more and to achieve controlled humidity of respired gas, which is important for the accuracy of the V_{O_2} computation heated and humidified gas was breathed from a 300 l neoprene bag (Camp Helsingborg Sweden) The inspired gas (air or N_2/O_2 mixtures containing about 14 and 30 per cent oxygen respectively) was supplied from pressure tanks at a rate sufficient to keep the inspiratory bag half filled during the experiments In the bottom of the bag there was approximately 10 l of water The water was heated by means of a brass tube through which water from a thermostating unit (Model FS Gebrüder Haake Berlin, FRG) was circulated A blower circulated the inspired gas through wide bore externally insulated respiratory tubing (i.d. 38 mm) and from a T-connection a *respiratory valve* (dead space 35 ml) was connected With this arrangement the inspired gas was saturated with water and, within narrow limits, kept at 37°C Both the respiratory valve the expiratory tubing and a *Venturi-type flowmeter* (Wigertz 1969) were heated by means of electrical heating tape (Electrothermal Engn Ltd London) in order to avoid condensation of water with ensuing volume changes of the expirate At the outlet of the flow meter a low-resistance manifold was connected to allow the collection of expired gas in 300 l neoprene Douglas bags The

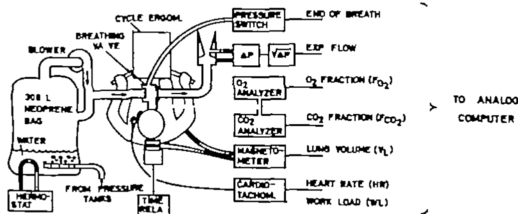


Fig. 2. General experimental arrangement. The inspiratory circuit is shown t the left of the subject sitting on a cycle ergometer whereas the various recording instruments are shown to the right The symbols ΔP and $\sqrt{\Delta P}$ denote a differential pressure transducer and a square-root amplifier respectively

pressure differential (ΔP) of the flow meter was measured using a differential pressure transducer (Model 270 with amplifier model 311 Sanborn Company Waltham Mass., USA) The ΔP of the Venturi flowmeter is proportional to the flow raised to the second power for which reason a square root amplifier was required to obtain a linear flow signal *A pressure switch* responding to the small pressure changes in the mouthpiece was used to detect the moment of termination of each breath. In the axial stream in the mouthpiece of the breathing valve a Teflon sampling tube was inserted (length 1.5 m i.d 1.3 mm electrically heated to about 60°C) A sampling flow of 1 l/min was drawn by a vacuum pump (Model EDM 2, Edwards Ltd., Crawley England) through the sensing heads of a linear infrared *CO_2 analyzer* (Capnograph CG 119 Godart De Bilt Holland) and a fuel cell *O_2 analyzer* (Model 209 Westinghouse Corp., Pittsburg Pa USA) The logarithmic output of the O_2 analyzer was linearized using an antilogarithmic preamplifier (Sonus AB Sollentuna Sweden) In the sampling line between the analyzers and the vacuum pump a constant-flow regulator (Model DK 46 Krohne Duisburg FRG) was connected *Lung volume* (V_L) changes were monitored from changes in the anteroposterior diameters of the thorax and the abdomen using magnetometers as described by Mead *et al.* (1967) In this method two exciter coils generating a magnetic field are attached at the level of the nipples and the umbilicus on the anterior surface of the body and two receiver coils are attached to the surface of the back at corresponding levels An increase in the antero-posterior diameter results in a decrease of the intensity of the magnetic field and thus a decrease in the voltage induced in the receiver coils In order to avoid interference between chest and abdominal magnetic fields, different exciter frequencies are used By displacement of the pulmonary gas content from chest expansion to abdominal expansion while breath-holding (isovolume maneuver), the ratio between chest and abdominal movements for the same volume change can be determined for each subject Using this ratio to obtain equal weights with respect to volume changes, the two signals are added to obtain a signal with a linear relationship to changes in thoraco-abdominal volume (Mead *et al.* 1967 Gilbert *et al.* 1972) *Heart rate* (HR) was obtained from precordial ECG leads using a beat-to-beat heart rate meter with a linear analog output (Lindborg Wigertz and Ödman 1969)

Calibration of Transducers

The flowmeter was calibrated by having dry air pass the meter at a known constant rate (using a rotameter) with correction being made for the difference in density and hence in signal output between the dry air of room temperature and expired gas at 37°C (Wigertz 1969) The O_2 and CO_2 analyzers were calibrated with dry gases from pressurized cylinders the gases having been previously analyzed according to Scholander's micro

method Since the expiratory circuit and the gas sampling line were heated to avoid condensation of water vapor and the analyzers have internal temperatures far exceeding the temperature of the expired gas, all volume and concentration measurements were made with gas having the same composition as during BTPS conditions, *i.e.* an amount of water vapor corresponding to the saturation pressure at body temperature was present to constitute about 6 per cent of the volume flow through the Venturi tube and correspondingly dilute the analyzed sample Since the O_2 and CO_2 analyzers are not sensitive to water vapor *per se* (Linnarsson and Lindborg 1974) the F_{O_2} and F_{CO_2} readings obtained reflect the actual volume fractions of O_2 and CO_2 in the expirate and thus, multiplied by the barometric pressure (P_B) yield the corresponding partial pressures It also follows that O_2 and CO_2 volumes that can be derived from the present volume and concentration measurements are to be expressed as volumes BTPD and multiplied by $P_B \times 273/$ $/760\,(273+37) = P_B/863$ yield the corresponding STPD values.

The magnetometer output was calibrated against a known tidal volume by having the subjects breathe back and forth from a small plastic bag (volume 1 4 l) starting from functional residual capacity (FRC) and with a known volume of air in the bag. A previously determined FRC in sitting position for each subject provided a base-line value for this procedure yielding a calibrated V_L signal

Analog On-Line Computations

An analog computer (PACE TR 48 Electronic Associates Inc Princeton, N.J., USA) was connected on-line during the experiments By means of standard circuits and operations (Carlson *et al.* 1965) several computational procedures, such as integration multiplication, peak value detection etc could be performed simultaneously as is outlined on the flow diagram in Fig. 3 The continuous outputs from the various transducers were thereby transformed to one calibrated value per breath for each of the following variables duration of each breath (T) pulmonary mid-volume ($\bar{V}_L$) end-expired values of F_{O_2} ($F_{ET_{O_2}}$) and F_{CO_2} ($F_{ET_{CO_2}}$), tidal volume (V_T) and the volumes of O_2 taken up ($V_{T_{O_2}}$) and CO_2 eliminated ($V_{T_{CO_2}}$)

The operation of a time integrator was started and terminated by the "end of breath" signal from the pressure switch, and thus a voltage was obtained proportional to breath duration Similarly the various other computations were started and terminated for each breath.

The maximal and minimum values for lung volume in each breath as measured with the magnetometer were detected with peak following circuits and the arithmetic mean of these two volume readings were computed to obtain $\bar{V}_L$ for each breath. End-expired

(peak) values of F_{O_2} and F_{CO_2} were also detected by means of peak following circuits in the analog computer

V_T V_{TO_2} and V_{TCO_2} during each breath were computed as described by Linnarsson and Lindborg (1974) The theoretical basis for these computations is the following. The volume of O_2 taken up during one breath is the difference between the inspired volume of O_2 $\int_0^T FI_{O_2}$ insp flow (t) dt and the expired volume of O_2 $\int_0^T FE_{O_2}$ exp flow (t) dt, where T is the duration of the breath FI_{O_2} is the inspired and FE_{O_2} is the instantaneous expired fractional concentrations of O_2 By making the assumption $\int_0^T$ insp flow (t) dt = $\int_0^T$ exp flow (t) dt (eq 1) the more convenient relationship $V_{TO_2} = \int_0^T$ exp flow (t) ΔF_{O_2} (t) dt (eq 2) is obtained Corresponding calculations for CO_2 yield V_{TCO_2} The assumption in eq 1 that inspired and expired volumes are equal is incorrect when $V_{TO_2} \neq V_{TCO_2}$ and the respiratory exchange ratio (R) thus deviates from 1.0 This relationship introduces a potential error which requires subsequent correction in the computation of V_{TO_2} but not of V_{TCO_2} as long as the inspired F_{CO_2} is negligible

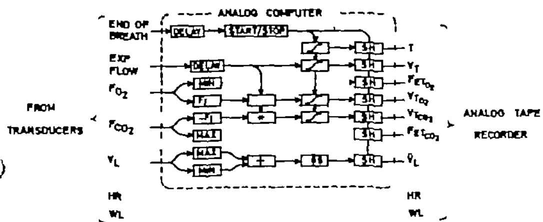


Fig. 3 Breath-by-breath on-line analog computations of (from above) duration of breath (T) tidal volume (V_T) end-tidal fractional concentration of O_2 (FET_{O_2}), volume of O_2 taken up per breath (V_{TO_2}), volume of CO_2 eliminated per breath (V_{TCO_2}), end-tidal fractional concentration of CO_2 (FET_{CO_2}) and pulmonary mid-volume ($\bar{V}_L$). The recordings of heart rate (HR) and work load (WL) are also indicated. The following symbols and abbreviations have been used to indicate the various steps in the computations. MIN detect minimum value, MAX = detect maximum value F_I = inspired fractional concentration − = subtract, + = add ☆ = multiply ∫ = integrate SH = sample hold. See Fig. 2 for explanation.

Signals from the rapid response transducers for flow F_{O_2} and F_{CO_2} were fed into the on-line connected analog computer The flow signal was electronically delayed some 100 msec in order to attain time-alignment to the concentration signals which were subject to a corresponding delay due to the transport lag in the sampling tube By comparing the instantaneous F_{O_2} and F_{CO_2} signals with preset $F_{I_{O_2}}$ and $F_{I_{CO_2}}$ voltage levels, voltages proportional to the differences between inspired and expired F_{O_2} and F_{CO_2} (ΔF_{O_2} and ΔF_{CO_2}) were obtained During expiration the expired flow and ΔF_{O_2} signals were multiplied, and the resulting signal integrated over the duration of the breath to obtain a voltage proportional to $V_{T_{O_2}}$ in analogy with the computation of expired V_T through integration of expired flow

The complete information for computing all the above variables was not available until a breath was terminated thereafter the computations were performed during 5–10 msec and the resulting values were held and recorded during the ensuing breath The output signals of the analog computer were recorded together with heart rate and work load on a *multichannel FM* tape recorder (*Model FR 100 C* Ampex Corp., *Redwood City Calif.*, USA, tape speed 1 7/8 i.p.s) and simultaneously displayed on a strip chart pen recorder (Mark 200 Brush Instruments Cleveland Ohio USA)

Calibration of the analog outputs for V_T (1 BTPS) and $V_{T_{O_2}}$ and $V_{T_{CO_2}}$ (1 BTPD) were obtained by adjustment of the gain factor of the integrators while supplying the flowmeter and the gas analyzers with gases of known flow and composition as described above (Calibration of Transducers) Impulses from a pulse generator with a frequency of 1/3 Hz were used to start and stop the computation of V_T $V_{T_{O_2}}$ and $V_{T_{CO_2}}$ every 3 sec during calibration

Digital Off Line Computations ☆

The stored data were subject to further off-line computations in a digital computer (IBM 1130) The play-back speed was four times the recording tape speed and A/D conversion (Interface 063 WDV Munich FRG) was made with a sampling frequency of 4/sec corresponding to 1 sec sampling intervals in experimental time

For each sampling interval a number of computations were performed as is schematically *outlined in Fig. 4 Barometric pressure* (P_B) for each experiment was used to convert $F_{ET_{O_2}}$ and $F_{ET_{CO_2}}$ readings to partial pressures and to compute a factor (P_B/863) to convert O_2 and CO_2 volumes from BTPD to STPD A value of $V_T/^?$ was subtracted from

☆ Detailed descriptions of the program software used in this study are available in the Library Karolinska Institutet, Solnavägen 1 S–104 01 Stockholm 60 Sweden.

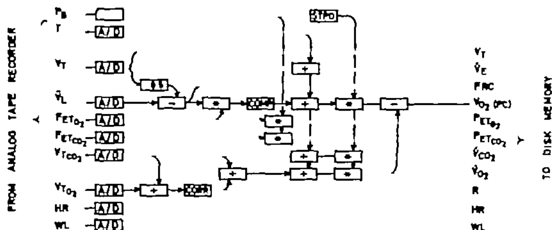


Fig. 4 Off-line digital computations using consecutive one-sec samples of the recorded variables (*cf* Fig. 3) The barometric pressure (P_B) for each experiment was also used for the computations. The following symbols and abbreviations have been used in addition to those listed in Fig. 3 to indicate the various steps in the computations: A/D analog to digital conversion, ÷ = divide STPD = = compute STPD conversion factor (p 15) COMP = compare previous sample CORR = compute correction factor for respiratory exchange ratio (p 18). V_T HR and WL undergo only A/D conversion. The new derived variables are (from above) ventilation (V_E) functional residual capacity (FRC), pulmonary capillary O_2 transfer ($V_{O_2(PC)}$) end-tidal partial pressures of O_2 ($P_{ET_{O_2}}$) and CO_2 ($P_{ET_{CO_2}}$) carbon dioxide elimination (V_{CO_2}) oxygen uptake (V_{O_2}) and respiratory exchange ratio (R).

pulmonary mid-volume to obtain *FRC*. *Respiratory exchange ratio* (R) was computed as $R = R\ (1 - F_{I_{O_2}})/(1 - R\ F_{I_{O_2}})$ where R is the ratio $V_{T_{CO_2}}/V_{T_{O_2}}$ having the above mentioned error in $V_{T_{O_2}}$ when $R \neq 1.0$ (Linnarsson and Lindborg 1974) A corrected $V_{T_{O_2}}$ was thereafter computed as $V_{T_{CO_2}}/R\ V_T\ V_{T_{CO_2}}$ and the corrected $V_{T_{O_2}}$ were divided by T to obtain V_E V_{CO_2} and V_{O_2}

In order to compute the *pulmonary-capillary O_2 transfer* $V_{O_2(PC)}$ (Auchincloss Gilbert and Baule 1966) the breath-by-breath changes of the pulmonary O_2 stores were subtracted from $\dot{V}_{O_2}$ as follows The product $F_{ET_{O_2}}$ x FRC for each sampling interval was computed and the corresponding value for the preceding sampling interval was subtracted This difference was divided by T to obtain the change in pulmonary O_2 volume per unit of time which in turn was subtracted from the simultaneous V_{O_2} value the result being $V_{O_2(PC)}$ Corresponding operations were performed to obtain *pulmonary-capillary CO_2 transfer* (not shown in diagram) The computed data were stored on a disk

memory and, in the case of the Protocol I experiments together with results from the two other identical experiments for subsequent computation of individual mean responses

Computation of Individual Mean and Group Mean Responses

Respiratory and circulatory functions exhibit spontaneous fluctuations which will be superimposed as a "noise" on the basic response pattern during e.g. the adaptation to exercise and become noticeable when breath-by-breath or beat-by-beat recordings are made. In order to reduce this noise and correspondingly enhance the basic response pattern repeated identical experiments were averaged both for each individual and for the whole group of subjects. The background noise is reduced by a factor of $\sqrt{n}$ where n is the number of determinations. In the case of group averaging also characteristic response patterns may be smoothed if they occur time scattered in relation to the stimulus in this study the change in external work load.

Individual mean time courses of V_{O_2}, $V_{O_2(PC)}$, $\dot{V}_{CO_2}$, R, V_E, V_T, FRC, P_{ETO_2}, P_{ETCO_2} and HR were computed using an IBM 1130 computer. For each subject condition and variable three on- and three off-responses were averaged the averaging procedure being synchronized to the work load changes, *i.e.* each sample of the individual mean response is the average of three corresponding values of the recorded responses. For the Protocol I experiments averaging was done with data from identical transients of three different experiments while for the Protocol II experiments averaging was done with data from the three identical transients in one experiment.

The individual mean responses were stored on digital magnetic tape and the further data processing was performed using a large scale computer (IBM 360/75).

Individual values for the final levels of V_{O_2}, $V_{O_2(PC)}$, V_{CO_2}, R, $\dot{V}_E$, V_T, FRC, P_{ETO_2}, P_{ETCO_2} and HR were computed as time averages over the last 30 sec of work and over the last 2 min of 0-load in each individual mean response. Mean values of these determinations are shown in Table III.

Group mean responses were computed from the eight individual mean-response data by the same principles as described above for the computation of individual mean responses. In this way a further reduction of random noise by a factor of $\sqrt{8}$ was obtained and at the same time the characteristic response patterns within the group were enhanced

Estimation of Parameters for Quantitative Description of Time Courses

In order to facilitate the comparison between the time courses of a variable under different conditions and also to make comparisons between different variables, the time courses were described in terms of mathematical functions that were fitted to the experimental data Since many of the variables under study (e.g V_{O_2} V_E HR) have been shown to behave like exponentially decaying functions of first or second order following step changes in external work load (Henry and De Moor 1956 Cerretelli Sikand and Farhi 1966 Broman and Wigertz 1971 Whipp and Wasserman 1972) such functions were fitted to the recorded responses. In the mathematical models the amplitude of a response was expressed as a function of time Thus the following functions of first order $f_1(t)$ and second order $f_2(t)$ were used

$$f_1(t) \begin{cases} 0 & \text{for } t < T_D \\ a_1\,[1 - \exp(-(t - T_D)/\tau_1)] & \text{for } t > T_D \end{cases}$$

$$f_2(t) \begin{cases} 0 & \text{for } t < T_D \\ a_1\,[1 - \exp(-(t - T_D)/\tau_1)] + a_2\,[1 - \exp(-(t - T_D)/\tau_2)] & \text{for } t > T_D \end{cases}$$

where a_1 and a_2 are amplitude coefficients, τ_1 and τ_2 are time constants and T_D is the time delay which for the second-order function is the same for both terms of the function (Fig 5) The amplitudes have the same dimension as the variable to be described by

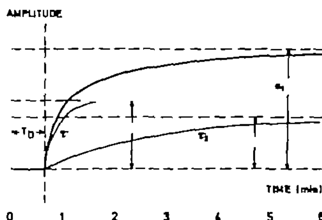


Fig. 5 Graphical representation of a second-order mathematical model employed to describe recorded responses, where the amplitud is expressed as a function of time (cf $f_2(t)$ p 20) 0-time indicates the time for a step change in work load The various function parameters used to characterize the response are indicated in the figure where T_D = time delay before the onset of the response τ_1 and τ_2 = time constants for each of the two terms of the function, a_1 and a_2 the corresponding amplitudes when t ∞ In this example T_D is 40 sec and τ_1 and τ_2 are 15 and 120 sec respectively

the function whereas τ_1 τ_2 and T_D are expressed in units of time One time constant is equal to the time when $(1 - e^{-1}) \times 100 \approx 63$ per cent of the final change has taken place For comparison the half time for a function is equal to $0.69 \times \tau$ and the term rate constant used by Whipp (1971) among others denotes the inverse of τ Ultimately when $t = \infty$ the change is equal to a_1 for the first-order function and to $a_1 + a_2$ for the second order function. For practical purposes it may be noted that after the slowest time constant has elapsed 5 times, only 0 7 per cent of the final change remains until the steady state value is reached In some of the second-order functions where the steady-state value was not reached during the 6–10 min of recording, $a_1 + a_2$ exceeded the difference between the final observed values of the on and off-responses listed in Table III

The computation of best-fit function parameters was made using a least-squares method *i.e* the function parameters were chosen so as to minimize the sum of the squared differences between the fitted function and the recorded response (loss function, L). To minimize L, an iterative algorithm was utilized in the present study The method was similar to that used by Broman and Wigertz (1971) and was further modified accord

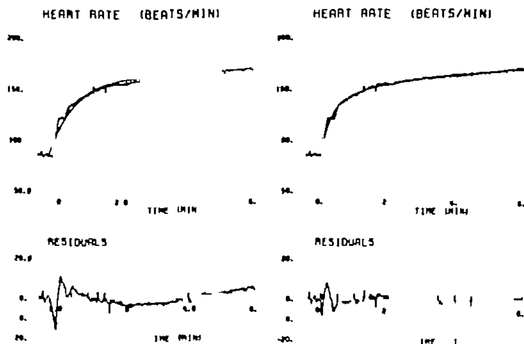


Fig. 6 Individual mean on-responses of heart rate to a step change in work load between 0-load and 240 W together with first-order (left) and second-order (right) best-fit functions. The superior fitting of the second-order function is clearly shown. Note notch in the heart-rate response approximately 10–20 sec after start of work. Residuals (lower tracing) denote the vertical distance between the fitted function and the recorded responses as a function of time

ing to Marquardt (1963) This fitting procedure can be carried out without knowledge of the final steady state value of a response and it operates efficiently even in the presence of noise and spontaneous fluctuations superimposed on the basic response signal For each variable experimental condition and subject and for the corresponding group averages mono- and biexponential functions were fitted to the recorded responses, starting 30 sec before a change in work load and continuing until the next shift in work load

An example of best fit mono- and biexponential functions is shown graphically in Fig 6 together with recorded data and the differences between recorded response and the fitted function as a function of time Certain criteria were used to judge whether an experimental response was better described by a second-order than by a first-order function a) The loss function had to be appreciably lower when the second-order model was applied b) In case a negative time delay resulted with the first-order model this delay had to be less negative with a second-order model c) The two time constants of the second-order model should be distinctly different d) Group averaging should reduce L approximately in proportion to the number of subjects by reducing random noise provided the applied model is a good description of the measured response If L is only slightly reduced during group averaging this implies that the order of the applied model is too low

Plotting of individual and group mean responses and best fit functions was done with a CALCOMP 835 plotter coupled to an IBM 360/75 computer and using standard subroutines

Computation of Mean Response Time O_2 Deficit and O_2 Debt

A simple measure of the rate of adjustment of a variable is the mean response time (MRT) For a first-order function this is the sum of the time delay (T_D) and the time constant (τ_1) The MRT value can be used for comparisons of the overall rate of change between responses to which first-order functions can be fitted

By definition the deficit for a response for example the O_2 deficit is equal to the area between the final or steady state level and the measured response throughout the work period Since in the fitting procedure the fitted curve follows the measured response so that the sum of areas between the two curves becomes zero the area between the final *level and the fitted function is also equal to the deficit The corresponding condition* holds true for the off responses Deficit and debt values can thus be obtained as the product of MRT and the amplitude of a response (Whipp 1971) when true steady-state values are reached during the recording period For those work loads where a true steady state was not attained the level of O_2 need used to compute the deficit was

defined as the mean value over the last 30 sec of work at each particular work load in agreement with the definition of O_2 deficit used by Hansen (1934) The O_2 need during exercise in hypoxia was defined as being equal to the final value of V_{O_2} obtained with the same work load during air breathing. In those recovery responses where the base-line V_{O_2} was not reached within 10 min debt was computed from the area between the best-fit function and base-line $\dot{V}_{O_2}$ until they converged.

Statistics

Conventional statistics were applied Intraindividual differences were evaluated using Student's *t*-test (Fischer 1946)

however the variable exhibited a continuous change throughout the measuring period although slower towards the end Therefore since all the values of Table III are not true steady state values, the term final value has been used.

Oxygen uptake during work increased linearly with the absolute work load in the graded AWL experiments On the basis of V_{O_2} values of the last 30 sec the relative work load (RWL, Table II) averaged 31 54 and 78 per cent respectively at 80 160 and 240 W The final values for V_{O_2} during air breathing in the Protocol II experiments (mean 2.57 l/min STPD) agreed closely with what could be predicted from the same average work load and using the $\dot{V}_{O_2}$/work-load relationship of the Protocol I experiments During the inhalation of 14 % O_2 in N_2 the final V_{O_2} value was significantly reduced by 6 per cent ($p < 0.01$) while no significant difference in V_{O_2} was observed between air and 30 % O_2 in N_2 Using the final value for $\dot{V}_{O_2}$ in the air experiments to determine the aerobic cost of work for each subject during all the three experimental conditions of the constant AWL study the mean relative work load (Table II) amounted to 64 per cent breathing air and to 80 and 60 per cent respectively with 14 % O_2 and 30 % O_2 During the last 2 min of 0-load V_{O_2} averaged 0.56 l/min STPD and did not vary with the preceding work load except after very heavy work, where 0-load $\dot{V}_{O_2}$ amounted to 0.61 l/min STPD

Carbon dioxide elimination during 0-load averaged 0 47 l/min STPD in the graded-AWL study whereas 0.03–0.07 l/min STPD lower values were observed in the constant AWL experiments

Respiratory exchange ratio during exercise increased with increasing relative work load to average about 0.97 with very heavy work and with hypoxia. During 0-load in the graded AWL experiments the lowest R values were found following the highest work load Zero-load R was consistently lower during the constant AWL than during the graded AWL experiments

Ventilation during exercise varied roughly in proportion to the relative work load from a mean value of 31 l/min BTPS at an RWL of 31 per cent to 93 l/min BTPS at an RWL of 80 per cent Corresponding changes were observed for tidal volume except for hypoxia where V_T was lower than during very heavy work despite largely the same average V_E

Functional residual capacity during work decreased with increasing RWL. During 0-load FRC averaged 3.30 l BTPS and did not differ between the various conditions.

End-expired P_{O_2} in all the air experiments averaged 102 mm Hg during exercise and was 2–6 mm Hg higher during 0-load Similarly with 30 % O_2 the $P_{ET_{O_2}}$ was higher during 0-load (172 mm Hg) than during work (165 mm Hg) With 14 % O_2 however 4 mm Hg lower values were obtained during 0-load where $P_{ET_{O_2}}$ averaged 62 mm Hg.

Mean end-expired P_{CO_2} during work ranged from 40 1 to 42 7 mm Hg in the air

breathing experiments and was slightly higher with 30 % O_2 With hypoxia P_{ETCO_2} was significantly lower by 9 mm Hg ($p < 0.001$) compared to air with constant AWL. Similarly P_{ETCO_2} during 0-load was significantly reduced with hypoxia ($p < 0.001$)

Exercise heart rate increased in an approximately linear fashion with absolute and relative work load in the graded AWL experiments. In the constant AWL experiments the highest mean value was found with 14 % O_2 while exercise HR did not differ significantly between air and 30 % O_2 and exceeded by 6 and 12 beats/min respectively the HR that would be predicted from the HR/RWL relationship of the graded AWL experiments. HR during the last 2 min of 0-load was increased after exercise at 240 W compared with corresponding values after 80 and 160 W In the constant AWL-experiments 0-load HR following a given RWL was 3–5 beats/min higher than in the graded AWL experiments.

Configuration of Responses

The group mean responses of V_{O_2} V_{CO_2} R, V_E V_T FRC P_{ETO_2} P_{ETCO_2} and HR are shown in Fig 7–13 Individual time courses have been recorded for each variable step response and condition. Essentially all the typical response patterns of the individual recordings could be found also in the group response recordings (see further "Intra and Interindividual Averaging" p 46) Close inspection of the curves reveals that the changes in HR started immediately whereas in the recordings of respiratory variables the first changes could be observed only after a few seconds delay which coincides with the inherent delays of the recording procedure

For $\dot{V}_{O_2}$ three distinctly different phases could be recognized both in the individual and in the group mean on-responses. Typically during the first 10–15 sec period there was a rapid increase followed by levelling-off during 5–10 sec at a level which for example in the 240 W experiments amounted to 1.2 l/min STPD Thereafter a rapid secondary change of large amplitude was observed being complete within 1.5–2 0 min. At work loads of 199 W and above a third phase resembling a slow upward drift could be discerned The corresponding three phases could be identified in the individual and group mean off-responses of V_{O_2} With the exception of a higher amplitude of the first phase the response pattern of $V_{O_2(PC)}$ agreed well with that of $\dot{V}_{O_2}$ (Fig 16)

$\dot{V}_E$ showed an initial rapid response of a relatively modest amplitude followed by a secondary change accounting for the major part of response This pattern could also be identified in the V_T recordings and in the on responses most of the initial change of V_T was accounted for by a rapid initial decrease in the FRC level

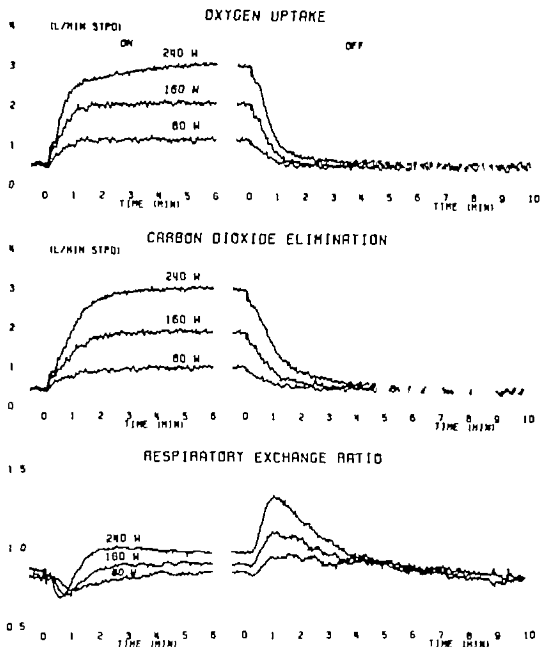


Fig. 7 Graphical representation of group mean responses of O_2 uptake, CO_2 elimination and respiratory exchange ratio to light (80 W), moderate (160 W) and very heavy (240 W) work, breathing air. Responses from both the transition between loadless pedalling and exercise (on) and the *reverse transition (off)* are shown. Each single tracing computed from breath-by-breath recordings from 24 experiments in 8 subjects. 0-time denotes the time for the change in work load.

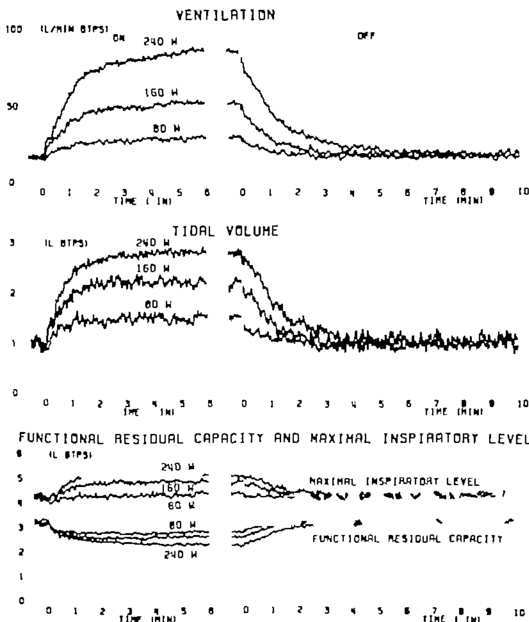


Fig. 8. Group mean on- and off-responses of ventilation, tidal volume and functional residual capacity to graded submaximal work, breathing air. Maximal inspiratory level is also shown, and was obtained by summing V_T and FRC responses. Same experimental conditions as in Fig. 7

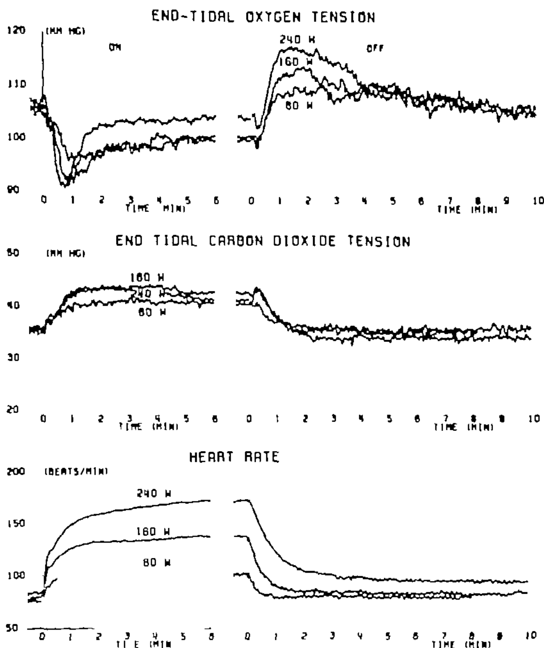


Fig. 9 Group mean on- and off-responses of end-tidal P_{O_2} and P_{CO_2} and heart rate to graded submaximal work breathing air. Same experimental conditions as in Fig. 7

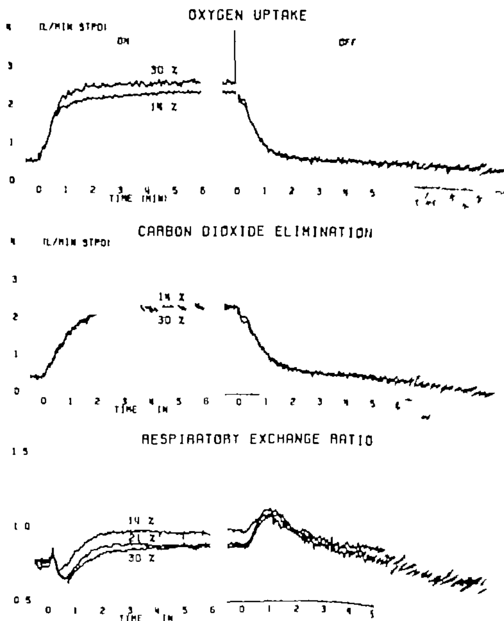


Fig. 10 Group mean on- and off-responses f O_2 uptake, CO_2 elim… ratio to exercise t work load demanding in each subject about 65 p… breathing. Tracings refer to experiments with 14 21 and 30 per cen… clarity data from the 21 % O_2 experiments have been omitted in th… single tracing computed from breath-by-breath recordings from 24 … denotes the time for step change in work load

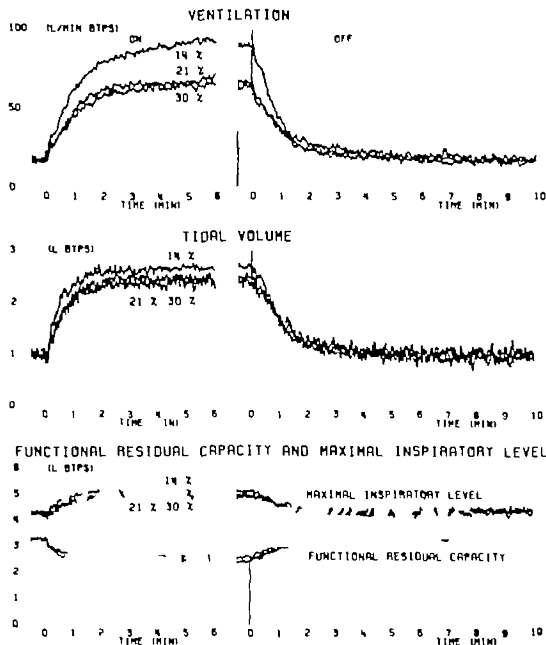


Fig. 11 Group mean on- and off-responses of ventilation, tidal volume functional residual capacity and maximal inspiratory level to constant submaximal exercise with hypoxia, normoxia and hyperoxia. Same experimental conditions as in Fig 10

END TIDAL OXYGEN TENSION

180 (MM HG) ON OFF
170
30 %
160
150
120
110
21 %
100
90
70
14 %
60
50
0 1 2 3 4 5 6 TIME (MIN)
0 1 2 3 4 5 6 7 8 9 10 TIME (MIN)

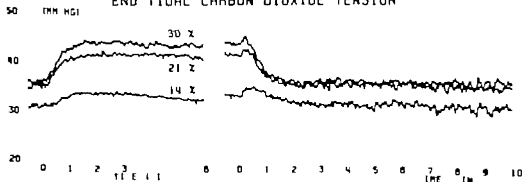


Fig. 12 Group mean on- and off-responses of end-tidal P_{O_2} and P_{CO_2} at constant submaximal work with hypoxia, normoxia and hyperoxia. Same experimental conditions as in Fig. 10

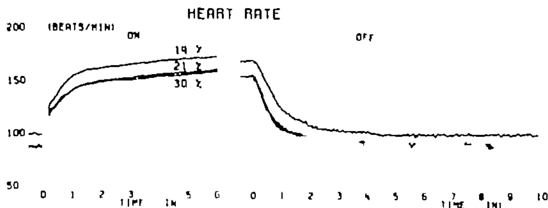


Fig. 13 Group mean responses of heart rate to constant submaximal work with hypoxia normoxia and hyperoxia Same experimental conditions as in Fig. 10

Parameterization of Responses

In order to express the configuration of the various recorded responses in quantitative terms and thereby allow comparisons and statistical analyses among the various time courses model functions were fitted to the recorded responses The object of this quantitation procedure has been primarily to describe the overall rates of change and to identify whether slow secondary changes appear in addition to the major initial responses (see further "Model Functions" p 46)

The fitting procedure and the criteria used to determine whether a first or a second order model comprised the best description of a recorded time course have been described above (p 22) Function parameters (amplitudes a_1 a_2 time constants, τ_1 τ_2 time delays T_D) of best fit functions for the various group mean responses are given in Table IV–VI For a first-order function the three parameters a_1 τ_1 and T_D are given and for a second-order function two additional parameters, a and τ_2 are included (*cf* Fig 5)

First-Order Functions were found to describe the time courses of respiratory variables adequately during the on and off-responses to exercise at the lower range of RWL Accordingly with light and moderate work and breathing 30 % O_2 (RWL $\leq$ 60 per cent) $\dot{V}_{O_2}$ and $V_{O_2(PC)}$ adjusted monoexponentially both in the on- and off-transients The V_E on responses to light and moderate work and with constant AWL breathing air or 30 % O_2 could be fitted with first-order models while this was the case for the off responses only with light work and with 30 % O_2 In these and other responses described as being of first order the use of a second-order model did not result in a better fitting between the measured responses and the applied functions

Table IV Parameterization (*cf* Fig. 5) of best-fit first or second-order functions for group mean responses of O_2 uptake pulmonary-capillary O_2 transfer CO_2 elimination and respiratory exchange ratio. Data refer to transients following onset of work (on) and recovery (off) at absolute work loads of 80 160 and 240 W breathing air (graded AWL) and at a constant AWL (group mean 199 W) performed at inspired O_2 concentrations of 14 21 and 30 % respectively (τ_1 τ_2 and T_D values given in sec).

		on					off				
		a_1	τ_1	a_2	τ_2	T_D	a_1	τ_1	a_2	τ_2	T_D
O_2 uptake, l/min STPD											
	80 W	0.47	29.4			9.3	-0.44	37.1			1.5
21 % O_2	160 W	1.56	32.5			8.1	1.57	37.8			3.6
	240 W	2.12	28.5	2.71	1980	7.8	-2.30	33.6	-0.23	710	9.9
14 % O_2		1.62	29.3	0.77	930	6.0	1.74	33.5	1.25	4140	12.2
21 % O_2	Constant	1.78	26.7	2.42	3800	10.9	1.99	38.1			5.2
30 % O_2	AWL	2.01	33.4			9.3	2.06	40.7			3.9
Pulmonary capillary O_2 transfer l/min STPD											
	80 W	0.89	28.1			1.5	-0.70	36.0			-7.3
21 % O_2	160 W	1.63	33.3			2.5	1.60	35.1			0.3
	240 W	2.01	31.2	0.69	220	-1	-2.16	27.0	-0.24	302	10.9
14 % O_2		1.44	23.8	0.46	148	5.8	1.70	29.0	2.41	8720	13.4
21 % O_2	Constant	1.85	31.2	2.55	4160	4.1	1.93	34.6			7
30 % O_2	AWL	2.13	34.2			1.9	2.11	38.6			3.0
CO_2 elimination, l/min STPD											
	80 W	0.55	53.4			5.6	-0.54	50.9			3.1
21 % O_2	160 W	1.48	52.8			7.4	1.35	48.5	-0.24	482	0.8
	240 W	2.59	54.6			9.1	2.39	62.2	-0.82	2590	2.9
1 % O_2		1.96	54.2			.8	82	42.6	1.15	3800	12.2
21 % O_2	Constant	1.87	53.0			7.9	1.80	53.3	-0.78	3880	2.9
30 % O_2	AWL	1.88	57.7			6.2	1.87	57.7			1.9
Respiratory exchange ratio											
	80 W	-0.62	43.1	0.60	5.9	8.5	0.3	95.1	1.63	5370	27.0
21 % O_2	60 W	1.34	20.7	1.32	29	34.6	0.31	19.8	-0.40	179	23.2
	240 W	2.88	16.3	3.05	20.3	21	0.48	21.7	-0.87	129	23.7
14 % O_2		0.22	30.1			49.2	0.24	17.7	-0.7	22	28.3
21 % O_2	Constant	.47	17.3	.53	24.5	20.9	0.1	8	-0.53	1.6	23.0
30 O_2	AWL	-0.47	14.3	0.55	40.3	21.2	0.29	4.7	-0.38	161	22

First-Order Time Constants ranging between approximately 20 sec and 70 sec were found For both on and off responses of $\dot{V}_{O_2}$ and $\dot{V}_{O_2(PC)}$ τ was about 30 sec $\dot{V}_{CO_2}$ changed at a considerably slower rate the τ_1 values being about 50–60 sec Corresponding values for $\dot{V}_E$ ranged between 60 and 70 sec

Table V Parameterization of best-fit functions for group mean responses of ventilation, tidal volume and functional residual capacity For symbols and experimental conditions, see Table IV

		on					off				
		a_1	τ_1	a_2	τ_2	T_D	a_1	τ_1	a_2	τ_2	T_D
Ventilation l/min BTPS											
	80 W	12.7	60.1			5.6	12.4	51.7			-6.0
21 % O_2	160 W	35.0	61.3			3.5	28.8	48.3	7.87	308	1.9
	240 W	63.3	62.3	42.3	3450	8.8	54.5	65.9	12.9	304	2.0
14 % O_2		61.7	58.8	22.6	425	2.2	-44.6	45.1	-41.2	3160	3.0
21 % O_2	Constant	51.2	61			2.6	-45.7	59.1	-6.6	348	-0.6
30 % O_2	AWL	48.8	69.6			1.0	-45.6	46.7			1.7
Tidal volume, l BTPS											
	80 W	0.51	31.3			9.3	-0.18	1.2	-0.32	41.0	1.3
21 % O_2	160 W	1.22	40.6			6.1	1.20	41.0			3.2
	240 W	1.82	44.5			4.4	1.77	78.7			9.0
14 % O_2		0.66	11.0	1.01	43.5	4.7	1.66	52.4			12.8
21 % O_2	Constant	1.49	44.2			1.9	1.48	70.5			5.7
30 % O_2	AWL	1.39	34.8			5.8	1.40	70.9			6.0
Functional residual capacity l BTPS											
	80 W	-0.30	14.5			3.1	0.36	43.3			5.0
21 % O_2	160 W	-0.57	24.1			3.1	0.62	63.			0.4
	240 W	-0.52	10.4	0.48	132	2.5	0.96	92.7			3.0
14 % O_2		-0.54	10.7	-0.3	77	4.3	0.87	72.6			4.3
21 % O_2	Constant	-0.34	5.1	-0.57	53.9	3.1	0.58	51.9	0.32	310	1.9
30 % O_2	AWL	-0.16	2.8	-0.55	38.1	4.3	0.49	74.9			1.3

Both *First and Second-Order Functions* could be fitted to the on and off responses of HR, to the $\dot{V}_{O_2}$ $V_{O_2(PC)}$ and V_L responses in the higher range of RWL and to the on responses of FRC For those responses the use of a second-order model resulted in a better fit between the measured time course and the applied function This is graphically shown in Fig 6 where an individual on-response of HR is shown together with best fit functions of first and second order For biphasic changes such as the on- and off-responses of R and $P_{ET_{CO_2}}$ the superior fit of second-order models was particularly evident and the L values frequently differed from those of the first-order fit by a factor of 10 or more

Second-Order Time Constants generally consisted of one rapid time constant dominating the initial part of the response and a slower component dominating the later part of the response τ values considerably exceeding the duration of the recorded on (360 sec) and the off (480—600 sec) responses are associated with a greater error in their determination compared with response components reaching their steady-state values within

Table VI Parameterization of best-fit functions for group mean responses of end-tidal P_{O_2} and P_{CO_2} and heart rate. For symbols and experimental conditions, see Table IV

		on					off				
		a_1	τ_1	a_2	τ_2	T_D	a_1	τ_1	a_2	τ_2	T_D
End-tidal O_2 tension, mm Hg											
	80 W	-20 6	41 9	22,3	81 9	7	9 9	22,5	-55,5	6490	20,5
21 % O_2	160 W	-43,2	21 8	37 8	39 4	19 2	16,	22,3	12,4	200	22 1
	240 W	-76	15,8	75,5	25,1	18,4	21 4	26,8	20,8	197	23 8
14 % O_2		-7 2	6,5	12 3	52,6	20,0	7,5	31 0	11,7	134	31,8
21 % O_2	Constant	-78,5	14,7	74,7	24 1	19 0	10,3	22 1	1 6	140	22 9
30 % O_2	AWL	23,8	16,3	27 9	46 9	19 0	16 6	10,6	-11,5	270	22,2
End-tidal CO_2 tension, mm Hg											
	80 W	5 0	34,5			1,8	2,5	8,3	3,0	104	21 4
21 % O_2	160 W	7,5	23,7			17,3	-7 4	30,1			25,2
	240 W	9 2	31,2	25 0	2150	13,0	-7 9	34 9			34 0
14 % O_2		4 4	43,5	11 1	1000	25 1	7,5	31 0	-11,7	136	31 8
21 % O_2	Constant	6,4	25 1			16,2	-6,1	22 4	12,7	7100	31,7
30 % O_2	WL	7,8	23,8			17 9	-8,3	28,3			28,1
Heart rate, beats/min											
	80 W	32,9	11,2	3 9	347	1,7	22,3	15 9			2,1
21 % O_2	160 W	49 7	22,4	44,6	1310	1,3	-51 6	25,5	-4 1	199	2,3
	240 W	57 6	20,3	34 7	143	1 0	-49 4	45 9	9 7	378	4,7
14 % O_2		57 1	26 8	49 2	851	-0,8	-56,8	44,2	12,8	107	7 3
21 % O_2	Constant	81,6	22,2	38 2	254	1 7	-45,7	27 0	20,1	113	3 1
30 % O_2	AWL	52,9	30,3	29 6	377	1,7	-54,7	31,7	10,0	253	2 9

the recording period Thus the slow components of *e.g* the V_{O_2} on responses to constant AWL and very heavy work breathing air ($\tau_2 \approx 2000$ sec or more) can just as well be described as being linear drifts having a more rapid rate for the higher work load For HR the rapid component had a time constant of about 20 sec followed by a slow component accounting for a larger portion of the response the higher the work load

Time Delays. Most responses to work-load changes took place after an initial delay ranging from a few sec to half a min. A short delay corresponding to the duration of one breath (approx. 2–3 sec) is inherent in the computation of the respiratory variables. For the heart rate recordings, the corresponding recording delay amounted to 0.5–1 0 sec

The fitting procedure may result in T_D estimates not found by close inspection of the recorded responses since T_D and the other function parameters from a mathematical point of view merely represent adjustment parameters to obtain best fit during the whole response period Especially in cases where the order of the selected exponential function is too low differences between estimated and recorded time delays may be found A small negative T_D may thus result when a rapid initial change has a large amplitude This

frequently occurred in the HR on-responses, and became in all cases much less pronounced when a second-order model was applied (*cf* Fig 6) In the same way the occurrence of an immediate change of only a small magnitude as in many respiratory variables and in the off responses of HR at high work loads may result in a short time delay in the fitted function.

The time delays of the order 15–30 sec found in almost all R $P_{ET_{O_2}}$ and $P_{ET_{CO_2}}$ responses are of such a magnitude that they cannot be ascribed to the recording or fitting procedures. Thus in these variables no or only slight changes took place during the first 15–30 sec following a change in work load

Mean Response Time (MRT for definition see p 22) represents the time until the "center of gravity" for a change has taken place and is synonymous with the term mean transit time commonly used in connection with dye dilution studies of blood flow Since the parameter MRT is an estimate of the overall rate of change for a fitted response it can be used to compare the responses between various variables and conditions (*cf* Table VII) In order to show the degree of dispersion of individual rates of response and to demonstrate the agreement between the means of individual and group mean responses, MRT values from group mean responses have been listed together with the means of individual determinations and their standard errors A good agreement was found between means of individual values and the MRT values of the group mean responses with *some few exceptions at the lowest work load The same good agreement was found for* the other function parameters when comparing means of individual parameters and the corresponding group mean values This may be taken to indicate that any of these two ways to characterize the "average" individual can be used The most rapid overall changes were found in the on-responses of HR during light work (MRT = 15 1 sec) and the slowest changes were found in FRC during recovery after very heavy work (MRT = = 91 7 sec)

Comparisons Between Overall On- and Off-Responses

The general tendency was that variables and conditions exhibiting a slow on-response also showed a slow off-response However with the exception of light work certain differences between the dynamic behavior of on and off-responses were noted for some of the variables, and the levels of significanse of intraindividual differences between on and off values for MRT are listed in Table VIII With 14 % O_2 MRT for V_{O_2} was prolonged by 7 sec in the off response For V_{O_2} no other differences were *found between overall* on

Table VII. Mean response times (sec) for on- and off-transients of cardiorespiratory variables. Data refer to submaximal exercise at absolute work loads (AWL) of 80, 160 and 240 W breathing air and at constant AWL (group mean 199 W) performed at inspired O_2 concentrations of 14, 21 and 30 %, respectively. Values from group mean responses (GMR) are given together with mean values (M) and standard errors of mean (SEM) of individual determinations in eight subjects for V_{O_2}, $V_{O_2(PC)}$, V_{CO_2}, $\dot V_E$, V_T, FRC and HR. For symbols, see Table III

			on							off						
			$\dot V_{O_2}$	$\dot V_{O_2(PC)}$	$\dot V_{CO_2}$	$\dot V_E$	V_T	FRC	HR	$\dot V_{O_2}$	$\dot V_{O_2(PC)}$	$\dot V_{CO_2}$	$\dot V_E$	V_T	FRC	HR
21 % O_2	80 W	GMR	38.7	27.4	59.0	45.7	40.8	21.6	15.1	38.5	28.7	45.8	46.7	26.2	38.4	10.0
		M	39.4	33.4	47.2	81.1	39.2	21.5	15.6	38.1	38.5	45.6	46.4	34.1	53.9*)	10.4
		SEM	2.4	4.1	11.9	19.1	6.8	5.8	1.6	1.4	9.3	4.5	7.4	8.7	20.7	1.4
	160 W	GMR	40.4	35.8	60.2	64.8	45.7	27.2	28.4	41.5	35.3	54.4	62.1	63.2	63.8	30.9
		M	40.4	35.7	68.4	65.1	45.7	31.3	28.9	41.3	34.9	54.3	64.0	61.9	66.9	31.8
		SEM	1.6	1.8	3.3	4.3	4.6	5.5	2.7	1.0	3.8	3.6	5.7	3.1	8.1	1.7
	240 W	GMR	45.4	41.9	63.6	74.2	48.9	42.3	39.7	44.0	42.2	70.8	78.4	87.7	91.7	60.2
		M	45.9	41.8	63.8	74.3	49.9	44.8	40.8	44.0	2.3	71.2	78.9	89.2	95.8	61.5
		SEM	1.4	1	2.1	3.1	6.9	8.8	2.9	1.0	1.7	3.5	3.1	5.9	8.6	3.5
14 % O_2	Constant AWL	GMR	1.8	40.0	59.0	74.4	33.7	29.1	48.0	48.7	44.1	60.0	57.2	65.2	76.9	59
		M	47.6	39.1	58.3	71.6	33.1	28.9	48.3	48.5	44.9	59.7	57.2	64.3	76.3	59.9
		SEM	1.3	1.8	2.4	8.3	2.4	5.3	2.8	1.5	2.2	3.0	3.0	2.5	6.0	8
21 % O_2		GMR	42.0	40.1	60.9	63.9	44.1	39.3	38.2	43.3	42.5	60.6	65.9	74.2	79.4	49.2
		M	42.3	37.7	40.6	60.2	45.0	39.9	38.1	43.2	42.1	60.0	65.0	75.9	76.7	8.8
		SEM	1.7	2.4	2.4	5.0	3.1	7.0	4.1	1.5	2.1	4.7	5.3	6.4	8.2	4.6
30 % O_2		GMR	42.6	36.1	63.8	70.6	40.5	33.8	33.1	44.6	41.6	59.6	45.0	78.8	76.1	42.9
		M	43.8	35.1	64.0	70.6	41.2	32.8	32.9	44.6	2.4	59.2	44.0	76.3	72.4	43.1
		SEM	1.7	1.4	3.3	3.3	3.7	7.4	3.9	1.6	3.2	4.2	4.9	9.9	11.2	4.8

) 7

Table VIII Levels of significance for differences between on- and off responses of $\dot{V}_{O_2}$, $\dot{V}_{CO_2}$, V_E, V_T, FRC, and HR. Data refer to submaximal exercise at absolute work loads (AWL) of 80 160 and 240 W breathing air and at a constant AWL (group mean 199 W) performed at inspired O_2 concentrations of 14 21 30 %, respectively 0 * ** and *** denote not significant $p<0.05$ $p<0.01$ and $p<0.001$ respectively For symbols, see Table III.

		$\dot{V}_{O_2}$	$\dot{V}_{CO_2}$	$\dot{V}_E$	V_T	FRC	HR
21 % O_2	80 W	0	0	0	0	0	0
	160 W	0	0	0			0
	240 W	0		0			
14 O_2	Constant AWL		0	0			
21 % O_2		0	0	0			
30 O_2		0	0	0			

Table IX. Levels of significance for differences between various experimental conditions, with regard to the mean response times for on- and off-transients of V_{O_2}, $\dot{V}_{CO_2}$ V_E, V_T, FRC and HR. Data refer to submaximal exercise at absolute work loads (AWL) of 80 160 and 240 W breathing air and at a constant AWL (group mean 199 W) performed at inspired O_2 concentrations of 14 21 and 30 % respectively For symbols, see Table III, VIII

		on						off					
		$\dot{V}_{O_2}$	$\dot{V}_{CO_2}$	$\dot{V}_E$	V_T	FRC	HR	$\dot{V}_{O_2}$	$\dot{V}_{CO_2}$	$\dot{V}_E$	V_T	FRC	HR
21% O_2	80 W 160 W	0	0	0		0		0				0	**
	160 W 240 W		0		0	0				**			
	80 W 240 W		0	0	0							0	
Constant AWL	14% O_2 21% O_2	0		0		0	0		0	0	0	0	
	21% O_2 30% O_2	0	0	0	0	0	0	0	0	0	0	0	0
	14% O_2 30% O_2	0	0	0	0	0	0		0		0	0	

and off-responses. In conditions where RWL averaged > 60 per cent the recovery of HR was prolonged compared to the on-response In contrast to V_E which did not differ in this respect between on and off V_T and FRC changed more slowly during recovery than following onset

Changes in Overall Responses with Different Work Loads and Inspired O_2 Tensions

In the experiments with graded AWL V_{O_2} did not differ between light and moderate work with respect to the overall rates of change in the on- and off responses (Table IX) By contrast MRT with very heavy work was significantly prolonged both in the on- and off responses of V_{O_2} due to the contribution of a second slower component to the response pattern V_E V_T and FRC responses seemed to be slowed under the influence of an increasing work load to a larger extent during the off responses than during the on responses When the RWL was varied by using a constant AWL and varying the inspired P_{IO_2} no consistent changes in the overall rates of on responses were observed despite changes in RWL of about the same magnitude as the difference between the two highest loads in the graded AWL experiments. During recovery however the rates of change of both HR and V_{O_2} were significantly slowed with hypoxia compared to normoxia and hyperoxia

V DISCUSSION

A Methodological Considerations

Maximal O_2 Uptake

Duplicate determinations of $\dot{V}_{O_2}max$ using the present technique have in this laboratory been shown to agree within 2 per cent at both normal and increased $P_{I_{O_2}}$ The changes in $V_{O_2}max$ obtained with hypoxia (−20 per cent) and hyperoxia (+7 per cent) in the present study agree well with previous findings (Fagraeus *et al.* 1973 Linnarsson *et al.* 1974) *from experiments where* $P_{I_{O_2}}$ *was altered by changing the ambient air pressure In the* present study particular care was taken to ascertain a constant and known $F_{I_{O_2}}$ both during maximal and submaximal work The recording of respired F_{O_2} allowed $F_{I_{O_2}}$ to be continuously monitored throughout the experiments. In addition $F_{I_{O_2}}$ was verified in samples drawn from the inspiratory part of the breathing valve and subsequently analyzed with the Scholander technique

Breath-by Breath Measurements during Submaximal Work

V_{O_2} V_{CO_2} and R The accuracy of the breath-by-breath measurements of V_{O_2} $\dot{V}_{CO_2}$ and R, using basically the present technique has been studied previously (Linnarsson and Lindborg 1974) and breath-by-breath determinations of V_{O_2} and V_{CO_2} were found to agree with simultaneous Douglas technique measurements within 0.02 l/min STPD during submaximal exercise In the present study some of the computational procedures previously performed with analog technique (division by breath duration computation of R) have been performed digitally which may have increased the accuracy of the computations slightly

Lung Volume Determinations of V_L using the magnetometer technique have been reported to agree well with simultaneous spirometric determinations (Mead *et al* 1967), Grimby Bunn and Mead 1968) FRC values determined during quiet breathing in the sitting position were used as a calibration base line Using body surface measurements to monitor respiratory mid-volume and to compute FRC by subtracting $V_T/2$ the absolute level of FRC is only properly determined if the nongaseous content of the thorax including blood remains constant There is clear evidence of a redistribution of blood volume from the legs towards the thorax at the transition from rest to exercise in the upright position (for review see Gauer and Thron 1965) but a corresponding decrease in residual volume of the lungs has not been observed (Asmussen and Christensen 1939) On the contrary Hanson Tabakin and Caldwell (1962) found a slight increase in total lung capacity

and residual volume in the transition from motionless standing to level walk. The same authors also found an unchanged residual volume comparing level walk with walking at 4° inclination. It is therefore concluded that the procedure used in the present study to assess lung volume changes adequately reflects the actual changes in FRC

End Tidal P_{O_2} and P_{CO_2} End-tidal P_{O_2} and P_{CO_2} are widely used as convenient indices of arterial blood gas tensions during exercise For both O_2 and CO_2 however end tidal estimates may differ from arterial determinations Such end-tidal to arterial differences may arise if a gas sample obtained at the end of expiration is not representative for the average composition of alveolar gas during a breath. This mechanism was proposed by Matell (1963) to explain the findings of lower end-tidal than arterial P_{CO_2} values during rest and the reverse difference during exercise in the following way During rest the alveolar sample is contaminated with dead-space gas while during exercise the increased metabolic rate and the forced breathing give rise to larger cyclic fluctuations of alveolar P_{CO_2} with the highest values at the end of the breath. For O_2 the same mechanism would lead to lower end tidal than mean alveolar values during exercise Mean alveolar O_2 tension may in turn appreciably exceed arterial values due to ventilation/perfusion differences. The interaction of these influences on the arterial to end tidal differences of P_{O_2} and P_{CO_2} has been analyzed by Rosenhamer (1972) and under conditions similar to the present experiments P_{aCO_2} during light exercise exceeded P_{ETCO_2} by approximately 2 mm Hg while P_{ETCO_2} was 1 mm Hg higher than P_{aCO_2} during moderate exercise It is therefore likely that the large differences in P_{ETCO_2} between 0-load and exercise in the present study to a large extent do not reflect corresponding differences in arterial P_{CO_2} For P_{O_2} end-tidal to arterial differences of 8–15 mm Hg were found for light and moderate exercise with P_{ETO_2} values closely approximating the P_{O_2} of an ideal alveolar gas sample

Computation of Pulmonary-Capillary O_2 and CO_2 Transfer A rapid increase in respiratory mid-level would increase the pulmonary O_2 store through two mechanisms, both because the volume of the alveolar space is increased and because an increased amount of inspired gas with a higher P_{O_2} is added It was thought of interest to determine to what degree changes in pulmonary O_2 stores were included in the O_2 uptake as measured at the mouth, and therefore the pulmonary-capillary O_2 transfer was computed breath by breath. Since the depth of inspiration is subject to irregular fluctuations in normal breathing (Hlastala Wranne and Lenfant 1973) a high level of "noise" will be introduced into a breath-by-breath recording of the O_2 stores and into a variable such as $V_{O_2(PC)}$ where changes in O_2 stores are included Although the typical response pattern for $V_{O_2(PC)}$ in the early portion of a response could be discerned despite the "noisy" character of recordings, it was not possible to establish whether or not secondary slow components of low am

plitude were present in individual recordings The $\dot{V}_{O_2(PC)}$ recordings therefore did not lend themselves to analysis of intraindividual differences in overall rates of response

From a theoretical standpoint breath by-breath changes in pulmonary CO_2 stores may be of minor importance for the time course of $\dot{V}_{CO}$ for two reasons First the volume of CO_2 in the alveolar space is very small compared to other rapidly changing CO_2 stores of the body (*cf* Farhl and Rahn 1955) Second changes in CO_2 volume and concentration due to a change in respiratory mid level tend to cancel each other Accordingly no differences were found between the recording of V_{CO_2} and pulmonary-capillary CO_2 transfer in a pilot study and this variable was not further studied

Experimental Conditions

Inspired O_2 Tensions. The inspired O_2 tensions in the present hypoxic and hyperoxic experiments were selected to create conditions similar to those in experiments by Linnarsson *et al.* (1974) who studied muscle metabolites during submaximal and maximal work and found that the O_2 deficit and lactate accumulation varied inversely with P_{IO_2} in the range of 100–200 mm Hg

Work Loads. Zero-load pedalling was used as a convenient base-line condition By having the subject pedal at the same rate during the entire experiment the work load could be changed without the subject s prior knowledge thereby avoiding anticipatory influences on the respiratory and circulatory response patterns. It was also thought that with the use of 0-load instead of rest between the work periods, the time needed for recovery could be shortened allowing a larger number of successive transients to be studied within the given period of time About one hour was found to be the longest experimental duration that could be used without causing any appreciable discomfort to the subjects.

The term zero load is a misnomer in the sense that a metabolic load is in fact imposed on the subject under 0-load conditions In the present study V_{O_2} during zero load averaged 0.56 l/min STPD Extrapolation from the AWL/V_{O_2} relationship in the 80 and 160 W experiments suggests that about 20 W is performed during 0-load which is considerably less than the value 45 W obtained by Beaver and Wasserman (1968) but agrees well with what can be calculated from data presented by Whipp and Wasserman (1969). The underlying assumptions for the above extrapolations remain however uncertain For the purpose of the present study 0-load is merely considered to be the mildest form of ergometric exercise possible with a pedalling rate of 60 rpm

The work intensities in the graded AWL experiments (80 160 and 240 W) were chosen to cover a wide range of submaximal work loads during air breathing A work load for each project corresponding to about 65 per cent of V_{O_2}max during air breathing was

selected for the constant AWL experiments. This work load was considered high enough to result in a significant lactate accumulation in the working muscles during air breathing (Karlsson 1971), and low enough to avoid a maximal work situation when the same external work load is performed in hypoxia where $V_{O_2}max$ is known to be decreased (*cf* Fagraeus *et al.* 1973)

For all subjects the relative work loads during air breathing were computed as the ratio between actual $\dot{V}_{O_2}$ during the last 30 sec of work and $V_{O_2}max$ The V_{O_2} values for submaximal work with air were considered to indicate the O_2 need for each particular work load and subject and accordingly RWL for the experiments with hypoxia was computed as the ratio between V_{O_2} during air breathing and $V_{O_2}max$ during hypoxia. Using the lower submaximal V_{O_2} values obtained during the hypoxic experiments would have given too low RWL values due to the slowed response of $\dot{V}_{O_2}$ during hypoxia.

Work Patterns. In recent years various work profiles such as sinusoidal step ramp function and impulse work-load changes (Stegemann 1958 Beaver and Wasserman 1968 Wigertz 1971 Fujihara Hildebrandt and Hildebrandt 1973a, b) have been used to study the dynamics of cardiorespiratory adaptation to exercise In the present study a step-change work-load pattern was used due to its simplicity both in regard to its application the subsequent analysis and the way the results can be directly visualized A six min work interval agrees well with what is commonly used in exercise testing and was chosen as a compromise between various considerations. Limitations in the data storage capacity on one hand, set a limit for the total time available for the experiments and on the other hand a certain number of repeated experiments was necessary to obtain the desired degree of noise reduction A third consideration was that the duration of each response must be long enough to allow proper determination of the dominating time components

Similar considerations were made also for the recovery period and it was also attempted to minimize the influence of one work period on the following by having the highest work load at the end of Protocol I (graded AWL) Adequate recovery of V_{O_2} was found 8 and 10 min after the 80 and 160 W experiments respectively and 10 min after work in the Protocol II experiments (constant AWL) A comparison between the HR before the onset of the 80 W work load and the 240 W work load (mean HR 76 and 84 beats/min respectively) on the other hand indicates a slight change with time in the base-line condition. For a given work period the relative intensity of the preceding work period had the highest values in the constant AWL experiments, notably those with hypoxia, where also the largest changes were found for base-line HR, mean values being 96 and 104 respectively during the last 30 sec of the first and third 0-load periods.

The R value at the end of a recovery period can also be used to indicate whether basal conditions of respiratory gas exchange have been achieved During severe exercise leading

to accumulation of fixed acids CO_2 stores in the body are mobilized and blown off from the lungs due to excessive hyperpnea Following the initial rise in R during recovery subnormal R values can be obtained when CO_2 stores are refilled This relationship has been illustrated by Hansen (1934) and may explain why recovery R values were slightly lower following repeated heavy exercise in the constant AWL experiments than following a comparable work load in the graded AWL experiments where only one very heavy work period was performed in each experiment

Parameterization and Data Reduction

Model Functions. Various mathematical models for the description of cardiorespiratory responses have been proposed and these have recently been reviewed by Fujihara *et al.* (1973b). Since the fitting of mathematical models to the recorded responses in this study was used to describe the overall responses rather than to detect all the involved time components, the simple model of Wigertz (1970) was used consisting of a time delay followed by one or two exponential changes starting at the same time This model has been proven useful also to detect the secondary slow changes in the responses (Broman and Wigertz 1971) which was one of the main objectives in this study

Intra- and Interindividual Averaging The theory underlying the averaging procedure has been presented above (p 19) Averaging of repeated individual respiratory responses to repeated identical stimuli has been used *i.a.* by Beaver and Wasserman (1968) Fujihara *et al.* (1973a b) and Reynolds and Milhorn (1973) in order to make the basic response pattern stand out more clearly It was found in these studies, that the basic features of each response were enhanced by the averaging procedure indicating that the response pattern was related in a constant way to the stimulus (work-load change) This was seen also in the present study when comparing the recorded and the averaged individual responses. One typical example is the characteristic "notch" in the HR on-response (Fig. 6) which remained apparent in the recordings before and after averaging The same HR pattern can also be used to illustrate the consequences of intraindividual averaging by comparing Fig. 6 and Fig 9 Evidently the temporal dispersion of this HR pattern within the group of subjects was small enough to make the individual pattern appear with only slight modification in the group mean responses. Similar conclusions both in regard to intra and interindividual responses can be made from other typical response features *e.g* the rapid initial drop in the FRC on response or the delay in the responses of R and P_{ETO_2} Therefore in the present study it has been considered justified to analyze both individual and group mean responses and since essentially all information from the individuals can be found in the group mean data, mainly the latter have been selected to illustrate the results.

Dynamic Behavior of $\dot{V}_E$ V_T and FRC in Response to Step Changes in Work Load

The ventilatory responses to exercise resembled those described by among others Asmussen and Nielsen (1948), Dejours (1959) and Matell (1963) showing an initial rapid response occurring without delay and followed by a secondary slower change starting some 20–30 sec later This response pattern was found uniformly in the subjects and was often found more distinctly in the off-responses of $\dot{V}_E$ (Fig 8 11) The responses to moderate and very heavy work differed from those reported by Dejours (1959) and Matell (1963) in that the magnitude of the initial component was smaller and less abrupt According to Dejours (1959) about half of the ventilatory response can be ascribed to the initial component but in the present study the average magnitude of the initial component exceeded 25 % of the final ventilatory level only during light work.

The possibility that important transients may not be evident in total ventilation but appear in V_T or respiratory rate has been pointed out by Dejours (1967) Accordingly V_T on- and off transients were studied In addition recordings of FRC were used to analyze whether changes in V_T were accomplished through changes in the inspiratory or expiratory reserve volumes (Fig. 8 11) In the on-responses V_T exhibited more distinctly the same basic response pattern as $\dot{V}_E$ *i.e.* a rapid initial component followed after about 20 sec by a secondary slower change There was a slight transient drop in V_T immediately before the appearance of the slower component The initial component could be accounted for almost entirely by a rapid drop in the FRC level This may be taken to indicate that the immediate respiratory response to the onset of dynamic leg exercise is an increase in expiratory activity At the same time the maximal inspiratory level remained almost unchanged (Fig. 8) According to Grimby Bunn and Mead (1968) a decrease in FRC is associated with a corresponding decrease in the abdominal diameter Since the abdominal muscles are abruptly engaged at the onset of dynamic leg exercise a simple mechanical relationship between leg and trunk movements may be of some importance for the initial changes in FRC level The secondary slower V_T increase mainly consisted of an increase in the maximal inspiratory level The final values of maximal inspiratory level and FRC (Fig 8 11) agree well with previous findings by Asmussen and Christensen (1939) that the increase in V_T is taken to a similar degree from the inspiratory and the expiratory reserve volumes

The overall response of V_T was markedly slower during recovery than following onset of work (Table VIII Fig. 8 11) and was associated with an equally slow time course of FRC towards pre-exercise values The difference in time course between the on and off changes in FRC suggests that the factors underlying the increased expiratory activity

effected through a withdrawal of vagal tone for example during isometric muscle contraction (Freyschuss 1970) In a recent study of the initial HR response to dynamic exercise (Fagraeus and Linnarsson 1974) it was found that also in this situation vagal withdrawal is the primary factor responsible for the rapid phase of the cardioacceleration Secondary to this rapid phase there is a further slow and continuous increase in heart rate (Broman and Wigertz 1971) with a concomitant slow increase in cardiac output (Rochester *et al.* 1961 Grimby Nilsson and Sanne 1966 Cerretelli *et al* 1966 Gilbert Auchincloss and Baule 1967) which is more marked at higher work loads (Jones *et al.* 1970).

The HR responses observed in the present study (Fig. 9 13) agreed largely with those described by previous investigators. Close inspection of individual and group mean curves reveals however that a more precise description of the on responses should include three components (1) a very rapid change during the first 10–15 sec (2) a slower rise during the following 60–90 sec and (3) a slow almost linear drift (Table VI) lasting throughout the work period In most individual HR recordings a levelling off or more often a temporary decrease in HR was observed following the initial rapid rise but before the secondary slower increase (Fig. 6 9 13) Such a negative component in the HR response can be discerned in the recordings of Craig, Cummings and Blevins (1963) and has also been described by Beaver and Wasserman (1968) and Fujihara *et al.* (1973a, b), who concluded that the "notch" in the on-response of HR is synchronized with the change in work load and is not merely a continuation of the sinus arrythmia of the base-line condition The notch in the HR response is abolished by atropine but not by β-adrenergic blockade (Fagraeus and Linnarsson 1974) which suggests that the initial vagal withdrawal during the first 10 sec is followed by a transient increase in vagal tone of about the same duration. The physiological mechanism underlying these biphasic changes in the vagal influence on the heart is not known

The magnitude of the initial component of the HR on response increased with the work-load in the graded AWL experiments (Fig 9) The share of the two slower components, however increased proportionally more from 30 per cent of the final 6 min-response with light work to about 50 per cent with very heavy work. This observation *which is in agreement with the findings of Broman and Wigertz (1971) implies that the* overall HR response is retarded with increasing work load (Table VII IX) With moderate and very heavy exercise the slowest HR component could be described as a linear drift amounting to 5 and 14 beats/min respectively during the last 4 min of exercise For comparison it may be noted that during clinical exercise testing HR is generally considered to have achieved a relative steady state if it does not change more than 10 beats/min from the second to the sixth min of exercise (Sjostrand 1967)

In the constant AWL experiments the on-responses of HR showed almost identical patterns with hypoxia normoxia and hyperoxia (Fig. 13 Table IX) In the case of

hypoxia however the response curve was shifted on the average 12 beats/min upwards (Table III) It thus seems that for the on-response of HR the rate of adjustment is deter mined by the absolute rather than the relative work load Hypoxia seems to cause a shift in the "set point level" for HR and this shift appears to be independent of AWL since it did not differ between 0-load and heavy work An increase in P_{ETO_2} of about 65 mm Hg induced by substituting 30 % O_2 in N_2 for air affected neither the rate of change nor the absolute levels of HR during the present experiments

The observation that the overall on response of HR became slower with increasing sub maximal AWL may at first sight seem to be at variance with the findings of Åstrand and Saltin (1961), that HR increases more rapidly the heavier the work load imposed during a maximal work test The findings of Åstrand and Saltin may however indicate that the amplitude of the rapid component of the HR response continues to rise with AWL also during supramaximal work, accounting for almost the whole change from base-line to maximal HR during extremely heavy work thereby leaving no room for the slower components

The off-response of HR did not exhibit any fluctuations of the type observed in the on-response but resembled closely a second-order exponential function (Fig. 9 13) The overall off responses were slower than the corresponding on-responses (Table VIII) which is in agreement with the observations of *Gilbert et al.* (1967) and Broman and Wigertz (1971) An interesting observation was that in the off-responses in hypoxia and very heavy work, the high HR persisted for some sec before the rapid drop occurred (Table VI) This phenomenon has been observed also in maximal exercise (Di Prampero Peeters and Margaria 1973).

The overall rates of change in the off-responses of HR were significantly slowed both with increasing AWL in the graded AWL experiments and with increasing RWL in the con stant AWL experiments (Table IX) which may be taken to indicate that the overall rate of the HR change during recovery is determined by the relative and not by the absolute work load the latter being the case in the ventilatory responses and the on-responses of HR. There is evidence that the metabolic situation in the muscles is involved in the regulation of the HR response to muscular exercise possibly through the action of peripheral receptors (Stegemann 1963 Asmussen *et al.* 1965 Stegemann and Kenner 1971) *The present results are compatible with such a hypothesis, since the metabolic changes* induced by varying the inspired P_{O_2} and thereby the RWL in constant-load experiments are likely to be much more pronounced at the end than immediately following the start of the exercise period This would explain why the rate of HR change at the start depends on AWL, while the corresponding rate of adjustment during recovery seems to depend on RWL.

Interrelation Between Ventilatory and Heart Rate Dynamics

A dissociated dynamic behavior of ventilatory and circulatory responses to muscular exercise has previously been observed (Wade and Bishop 1962 Wigertz 1970 Broman and Wigertz 1971) Such a dissociation would be expected to cause disturbances in the balance between the delivery of O_2 to the lungs and the removal of O_2 from the lungs by circula tion with ensuing changes in alveolar gas composition Also in the present study marked differences between the overall rates of change were noted between V_E and HR (Table VII) During the first minutes following a change in work load large transient changes were noted for $P_{ET_{O_2}}$ (Fig 9 12) indicating a transient relative hypoventilation in early exercise and a transient relative hyperventilation during recovery However during the initial 15–30 sec following the start or end of work the end tidal gas tensions remained

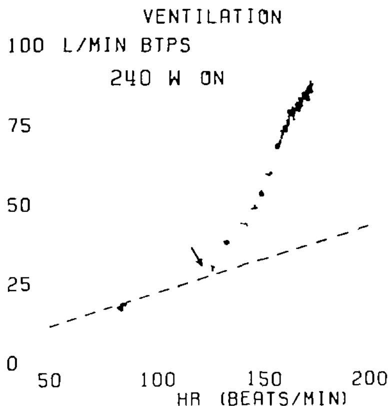


Fig. 14 Relationship between group mean responses of ventilation and heart rate following start f exercise at a work load of 240 W Small dots represent consecutive one-sec samples with each 30-sec sample indicated by a circle. The cluster of dots at bottom left refers to the last 30 sec f the base-line condition prior to the onset of work Arrow indicates when the first 15 sec of work have elapsed. Broken line indicates equal changes of the two variables in per cent of their base-line values.

relatively unchanged A remarkable constancy during the first 30 sec of exercise has been observed also in continuous measurements of arterial blood gases and pH (Matell 1963 Barr *et al* 1964 Bjurstedt and Wigertz 1971) The ventilatory and circulatory changes occurring during this interval thus seem to be fairly well synchronized which also became evident when the changes relative to the base line condition for V_E and HR were compared Thus both V_E and HR 15 sec after the onset of work at a load of 240 W comprised about 150 per cent of the base-line value This relationship is illustrated in Fig. 14 where group mean responses of V_E and HR have been plotted second by second in an x-y diagram It is therefore tempting to postulate a common regulatory mechanism for $\dot{V}_E$ and HR during the initial phase of exercise Since a change in the FRC level seems to account for the rapid initial phase of ventilatory response (Fig. 8) group mean FRC and HR changes were compared in an x y diagram (Fig 15) The rectilinear relationship in the diagram indicates almost identical time courses which suggests a common regulatory mechanism

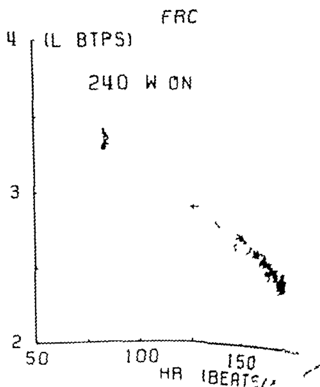


Fig. 15 Relationship between group mean responses of [illegible] art rate (HR) at the onset of exercise [illegible] work load of 240 W [illegible] values are shown at top left.

Dynamic Behavior of V_{O_2} in Response to Step Changes in Work Load

A survey of some earlier studies on the time courses of V_{O_2} following the onset and end of muscular exercise has been given above (see Introduction p 7) The present study is mainly concerned with two aspects of the dynamics of V_{O_2} first to describe as accurately as possible the various components of the time course of V_{O_2} in the unsteady state and second to relate the time course of V_{O_2} to the absolute and relative work levels and to the time courses of some other physiological variables

Of the three phases which can be distinguished in the time course of V_{O_2} in the unsteady state of exercise (p 27 Fig. 7 10) *the first phase seems to be related to the rapid* change in blood flow through the lungs that takes place immediately upon a change in work load This relationship between circulation and O_2 uptake was pointed out by Krogh and Lindhard (1912 1913) but it was not until half a century later that the initial rapid increase of the pulmonary-capillary O_2 transfer at the onset of work was actually recorded by Auchincloss, Gilbert and Baule (1966) These authors, who used an advanced computer-oriented technique to measure V_{O_2} breath by breath and to compensate for changes in pulmonary O_2 stores also showed that variations in the breathing pattern following onset of work do not greatly alter the time course of $V_{O_2(PC)}$ the pulmonary O_2 stores providing a buffer between the O_2 intake to the lungs and the pulmonary-capillary O_2 transfer Since the subjects in the present study exhibited a good synchronization between the initial responses of V_E and HR (Fig 14) V_{O_2} and $V_{O_2(PC)}$ displayed similar time courses in early exercise The magnitude of the initial rapid component of $V_{O_2(PC)}$ amounted to 0 85 l O_2 per min at the onset of work at a load of 240 W (Fig 16) com-

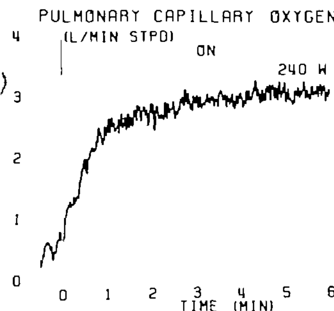


Fig. 16 Group mean response of pulmonary-capillary O_2 transfer at the onset of exercise at a work load of 240 W during air breathing. Tracing computed from breath-by breath recordings from 24 experiments in 8 subjects.

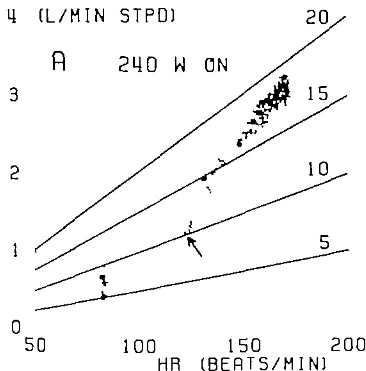
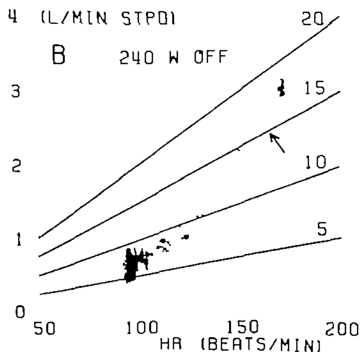


Fig. 17 *A* Relationship between group mean responses of pulmonary-capillary O_2 transfer and heart rate following the onset of exercise. O_2-pulse isopleths (ml O_2 per heart beat) are indicated. Dots, circles and arrow as in Fig. 14



B Relationship between group mean ff-responses f same variables as in *A*. Note that start values appear at top right.

prising about 30 per cent of the final 6 min response The interrelationship between the time courses of $V_{O_2(PC)}$ and HR is depicted in an x-y diagram in Fig. 17 (*cf* Davies 1968) where also O_2-pulse isopleths have been included During the first 15-sec period changes in stroke volume and/or in the arterial to mixed venous O_2 difference resulted in a relatively modest increase in the O_2 pulse from about 7 to 10 ml O_2 per heart beat Since some increase in stroke volume can be assumed to take place during the first 15 sec following the start of work in the upright position (Saltin *et al.* 1971) only slight changes in the $(a-\bar{v})$ O_2 difference are likely to have taken place during this period The temporary relative constancy of the $(a-\bar{v})$ O_2 difference may be characteristic only for the transition between 0-load and exercise or for the transition between different work loads, since Edwards *et al.* (1972) observed that mixed venous P_{O_2} ($P\bar{v}_{O_2}$) exhibited an early pronounced fall being almost complete within the first 15 sec following the transition from motionless rest to dynamic leg work of moderate intensity These authors ascribed the fall in $P\bar{v}_{O_2}$ to a sudden surge of blood with a low O_2 saturation from stagnant pools in the legs due to the activation of the muscle pump No such pools are likely to have occurred in the present study where 0-load pedalling was used as the base-line condition which may account for the apparent discrepancy between the present results and those of Edwards *et al.* (1972) The body position may also affect the time courses of the various adjustments following start and end of work Thus in the supine position the stroke volume has been reported to show a transient decrease immediately following the start of work (Raynaud *et al* 1973) and a transient increase during recovery (Cumming 1972) It can therefore be concluded that the greatest magnitude of the first phase of the $\dot{V}_{O_2(PC)}$ response should be expected in the transition from motionless rest to exercise in the upright position, whereas the corresponding response should be expected to be of considerably smaller magnitude in the transition between 0-load and work in the supine position

The rapid change in the O_2 pulse observed 15 sec after the onset of work (Fig. 17) corresponds to the *second phase* in the $\dot{V}_{O\ (PC)}$ and $\dot{V}_{O_2}$ responses Since most of the stroke volume change can be assumed to have taken place during the initial 15 sec (*cf* Saltin *et al.* 1971) a rapid widening of the $(a-\bar{v})$ O_2 difference is likely to be the cause of the further increase in O_2 pulse The delay until the widened $(a-\bar{v})$ O_2 difference caused an increase in $V_{O_2(PC)}$ presumably resulted from the time required for the transport of blood from the exercising muscles to the lungs The second phase of the $V_{O_2(PC)}$ and $\dot{V}_{O_2}$ responses was found to be completed within 1.5–2 min following the start of work presumably corresponding to the time necessary for the redistribution of blood flow to and within the working muscles Support for this assumption can be found in the results of Clausen and Lassen (1971) who found by the use of intramuscular depots of radioactive xenon that the increase in blood flow in the exercising muscles was completed within the first min of exercise

During light and moderate work steady-state values of $\dot{V}_{O_2}$ and $V_{O_2(PC)}$ were attained when the second phase was completed *i.e.* about 1.5–2 min after the start or the end of work. Fitting a first-order model to the data obtained during light and moderate work, a mean response time for V_{O_2} of about 40 sec was obtained corresponding to a "half time" of 28 sec which is in agreement with previous observations (Henry and De Moor 1956 Margaria *et al.* 1965 Cerretelli *et al.* 1966 Di Prampero *et al.* 1970 Whipp 1971 Whipp and Wasserman 1972) The corresponding value for $V_{O_2(PC)}$ was 5–10 sec shorter indicating that some 0.1 1 O_2 was drawn from the pulmonary O_2 stores at the start of work and then repaid during recovery In subjects with a sluggish V_E response the changes in pulmonary O_2 stores may be greater and it may then not be possible to discern two phases of the $V_{O_2(PC)}$ response from breath-by-breath recordings of V_{O_2}

During heavy and very heavy work there was a continuous slow change in $\dot{V}_{O_2}$ (*third phase*) also after the second min of exercise (Fig. 7). With these work loads the $\dot{V}_{O_2}$ responses could be described as second-order functions with one short time constant describing the first two phases of the response (rapid components) and one much longer time constant describing the slow component (Table IV) The short time constant was

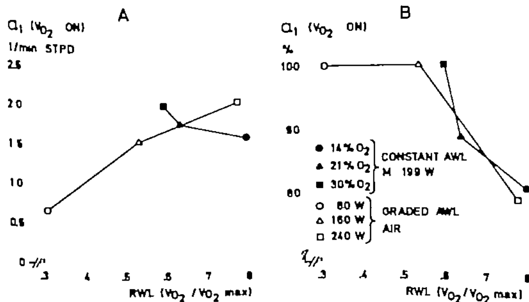


Fig. 18 *A* Amplitude (a_1) of the short time constant of the $\dot{V}_{O_2}$ on-response (*cf* Table IV) as a function of the relative work load (RWL). Not that 1 increases with the bsolute work load (AWL) but decreases for constant AWL when RWL is increased by hypoxia. For symbols, see *B*.

B a_1 in per cent of the O_2 need (*cf* p 26) as a function of RWL. Note that for RWL values exceeding bout 0.6 the relative contribution f the rapid time constant was progressively reduced both with increasing AWL values and when F_{IO_2} was lowered t a constant AWL, averaging 199 W

the discrepancy between the metabolic need and the O_2 delivery the resulting, gradually developing lactate accumulation or related metabolic change providing an "error signal for a further slow adjustment in the O_2 transport

The aforementioned observation that V_{O_2} during the 6th min of exercise was lower in hypoxia than in air indicates that in the former condition no steady state level of O_2 delivery was attained In contrast under experimental conditions where a true steady state can be obtained during exercise in hypoxia heart rate and cardiac output have been shown to increase sufficiently to compensate for a reduced arterial O_2 content so that the arterial transport of O_2 equals that during normoxia (Hughes *et al* 1968)

The dynamic behavior of V_{O_2} in response to on and off-changes in work load may be expressed in terms of O_2 deficit and O_2 debt These parameters reflect the overall rate of response and also to what extent steady-state levels of $\dot{V}_{O_2}$ are attained In Fig. 19 O_2 deficit and O_2 debt both for the graded and the constant AWL experiments have been related to the relative work level (*cf* Knuttgen 1970 Knuttgen and Saltin 1973) The O_2 deficit increased both with the absolute and the relative work load The O_2 debt also increased with AWL and RWL, and debt and deficit values agreed closely except in hypoxia where the large deficit was accompanied by a lower O_2 debt The observation that the

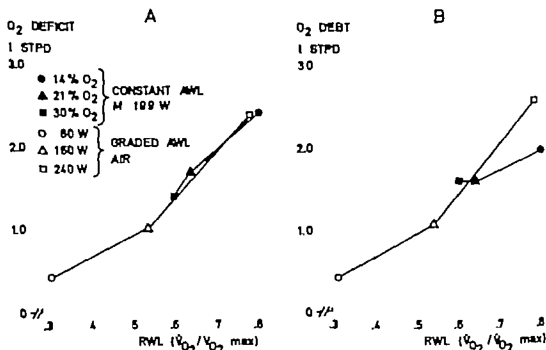


Fig. 19 *A* O_2 deficit as a function of relative work load.

B O_2 debt as a function of relative work load, for symbols, see *A*

debt/deficit ratio was equal to or less than unity with heavy and very heavy work differs from the classical concept that the repayment of the lactic O_2 debt occurring after work demanding more than 2/3 of $\dot{V}_{O_2}max$ (Margaria Edwards and Dill 1933) is associated with a debt/deficit ratio greater than unity (Asmussen 1946 Christensen and Högberg 1950, Henry and De Moor 1950) A tentative explanation for this discrepancy in results may be found in the fact that in the present experiments light work (0-load) was per formed during the recovery periods which would allow lactate to be oxidized as a substrate (Jorfeldt 1970 Hermansen and Stensvold 1972) This way of eliminating lactate would be associated with no extra O_2 cost in contrast to gluconeogenesis which is an energy demanding route of lactate elimination. The magnitude of the O_2 debt is thus influenced by the relative contributions of these two metabolic pathways for lactate elimina tion (*cf* Whipp Seard and Wasserman 1970) Furthermore the ability to oxidize lactate as a substrate has been shown to be enhanced during a given work period when preceded by prolonged heavy exercise (Karlsson 1971) This may have influenced the pathway of lactate elimination and hence the O_2 debt particularly in case of the present hypoxic ex periments, where the relative work load was higher than in any other experimental condi tion and furthermore the work was performed repeatedly

Dynamic Behavior of V_{CO_2} and R in Response to Step Changes in Work Load

The overall responses of $\dot{V}_{CO_2}$ were in all conditions considerably slower than those of V_{O_2} This is in agreement with previous observations by Robinson (1938) Cerretelli *et al* (1966) and Edwards (1969) The tissue stores for CO_2 appreciably exceed those for O_2 (Farhi and Rahn 1955) and it has been suggested that the buffering action of large peri pheral CO_2 stores explains why changes in mixed venous P_{CO_2} occur less rapidly than the corresponding P_{O_2} changes during onset of exercise (Edwards *et al.* 1972) Until the time when blood of altered composition due to a change in work load reaches the lungs the changes in V_{CO_2} will depend mainly on changes in cardiac output in the same way as V_{O_2} and $V_{O_2(PC)}$ Accordingly R exhibited only minor changes during the initial 15–20 sec period after a change in work load and then showed a transient drop during exercise (*cf* Davies 1968), and a transient overshoot during the first minutes of recovery (Fig. 7 10) The more pronounced overshoot during recovery from very heavy exercise (*cf* Hansen 1934) indicates that CO_2 is driven off from the body in excess of the metabolic production due to the accumulation of fixed acids, notably lactate

VI GENERAL SUMMARY

The time courses of O_2 uptake pulmonary-capillary O_2 transfer ($\dot{V}_{O_2(PC)}$) and ventilatory functions at start and end of exercise were studied on a breath-by-breath basis together with that of the heart rate Responses to step changes in work load formed the basis for the subsequent identification and quantitation of time components using a computer-oriented technique Experiments were carried out on 8 subjects performing light (80 W) moderate (160 W) and very heavy exercise (240 W) on a cycle ergometer in the sitting position breathing air and with zero-load pedalling serving as the base line In these experiments changes in the dynamic behavior of pulmonary gas exchange and heart rate were studied with respect to the effects of variations in absolute work load In a second series of experiments a heavy work load was used constant for each subject during hyperoxia (30 % O_2) normoxia (air) and hypoxia (14 % O_2) corresponding to relative work intensities of 60 64 and 80 per cent respectively of the maximal aerobic capacity By comparing the results from the two studies the recorded time courses could be related both to the absolute and to the relative work loads.

Dynamics of O_2 Uptake in Response to Light and Moderate Work

The adjustments of $\dot{V}_{O_2(PC)}$ during light and moderate work were completed within 2 min after the start and end of exercise and the time courses could be described as monoexponential functions with a time constant approximating 35 sec for both the on- and off responses in the two conditions. Close inspection of individual and group mean responses revealed however that a more precise description of the $V_{O_2(PC)}$ response should include two phases (a) a rapid initial change during the first 15–20 sec followed by (b) a second rapid rise lasting until about 1.5–2 min had elapsed after the change in work load *The first phase which presumably resulted from rapid changes in blood flow* through the lungs, accounted in the on-responses for 50 and 35 per cent of the final $\dot{V}_{O_2(PC)}$ change during light and moderate work, respectively The second phase presumably reflected changes not only in cardiac output but also in the arteriovenous O_2 difference the latter change appearing in the lungs only after a 15–20 sec delay corresponding to the time required for the transport of blood from the working muscles This interpretation is supported by the observation of a relative constancy of O_2 pulse end-expired P_{O_2} and P_{CO_2} and respiratory exchange ratio during the initial 15–20 sec period after a change in work load

The responses of $\dot{V}_O$ were in general slower than those of $V_{O_2(PC)}$ during the first

15–20 sec following a change in work load but thereafter the two time courses agreed closely Thus the overall rates of response expressed as mean response times (MRT) were 5–10 sec longer for V_{O_2} than for $\dot{V}_{O_2(PC)}$ indicating that some 0 1 l O_2 was drawn from the pulmonary O_2 stores at start of exercise and then repaid during recovery

Dynamics of O_2 Uptake in Response to Heavy and Very Heavy Work

The basic step responses of $\dot{V}_{O_2(PC)}$ with a time constant of about 35 sec were present also during unsteady state of very heavy work However the magnitude of the rapid changes during the initial phases of the on responses was not sufficient to meet the need for O_2 and consequently $\dot{V}_{O_2(PC)}$ continued to increase slowly throughout the 6-min work period The $V_{O_2(PC)}$ responses in very heavy exercise could thus be better de scribed as second-order functions the shorter time constant accounting for about 80 per cent of the final 6th min response Also during heavy work in air and in hypoxia the appearance of a slow component required second-order models to describe the $V_{O_2(PC)}$ responses whereas in hyperoxia the $V_{O_2(PC)}$ response to the same heavy work load could be described as a first-order function. Thus, an increase in relative work load (RWL) was accompanied by similar changes in $V_{O_2(PC)}$ response regardless of whether the increase in RWL was effected by increasing the absolute work load or by the inhalation of a hypoxic gas mixture at a constant preset load This indicates that the time course of $V_{O_2(PC)}$ is determined by the rate of acceleration of the circulatory O_2 transport and not by any inertia of the peripheral processes of O_2 utilization

A similarity between on- and off-responses of O_2 uptake was observed also in heavy and very heavy work, except during hypoxia As a consequence O_2-debt and O_2-deficit values agreed closely in any given condition except in hypoxia where the O_2 debt was slightly lower than the O_2 deficit

Dynamics of Ventilation and Heart Rate Changes

The ventilatory on and off responses were both found to consist of two distinctly dif ferent phases. The first phase of the on response was primarily due to a rapid increase in tidal volume brought about by a reduction of the functional residual capacity the second and slower phase occurring with a delay of 15–20 sec, *i.e* at the time when blood from the working muscles can be assumed to have reached arterial or medullary chemorecep tors The overall rate of change of V_I at the onset of work differed only slightly between the various conditions with MRT values approximating 70 sec During recovery however

the rate of change of V_E was markedly slowed with increasing absolute work load whereas no such slowing occurred when RWL was increased by hypoxia.

In general the overall heart rate responses were more rapid than the corresponding ventilatory responses. At the onset of work HR showed a very rapid initial increase followed by a short-lasting decrease or plateau before a second rise occurred With higher work loads the heart rate like V_{O_2} showed a slow component in both the on- and off responses which could be described by second-order models The response of HR at the start of exercise seemed to depend on the absolute work load the MRT values ranging from 15 sec during light work to 40 sec during very heavy work. During recovery the HR responses generally were slower than the corresponding on responses and became slower the higher the relative work load

VII REFERENCES

ASMUSSEN E., Aerobic recovery after anaerobiosis in rest and work. *Acta physiol.scand.* 1946 *11* 197–210

ASMUSSEN E., Muscular exercise. In *Handbook of Physiology Respiration*, edited by W O Fenn and H Rahn. Washington, D.C.. Amer Physiol. Soc. 1965 Sect. 3 Vol. II Chapt. 36 939–978

ASMUSSEN E., Ventilation at transition from rest to exercise. *Acta physiol. scand* 1973 *89* 68–78

ASMUSSEN E. and E.H CHRISTENSEN Die Mittelkapazität der Lungen bei erhöhtem O_2-Bedarf *Skand. Arch Physiol.* 1939 *82* 201–211

ASMUSSEN E. and M. NIELSEN Studies on the initial changes in respiration at the transition from rest to work and from work t rest. *Acta physiol. scand.* 1948. *16* 270–285

ASMUSSEN E., S.H JOHANSEN M JORGENSEN and M. NIELSEN On the nervous factors controlling respiration and circulation during exercise *Acta physiol. scand.* 1965 *63* 343–350

ÅSTRAND P-O and B SALTIN Oxygen uptake d ring the first minutes f heavy muscular exercise. *J appl. Physiol* 1961 *16* 971–976

ÅSTRAND P-O T.E. CUDDY B SALTIN and J STENBERG Cardiac output during submaximal and maximal work *J appl Physiol.* 1964 *19* 268–274

AUCHINCLOSS J.H J R. GILBERT and G.H BAULE Effect of ventilation on oxygen transfer during early exercise. *J appl. Physiol.* 1966 *21* 810–818.

BARKER, S.B. and W.H SUMMERSON The colorimetric determination of lactic cid in biological material. *J biol. Chem.* 1941 *138* 535–554

BARR, P-O M. BECKMAN H. BJURSTEDT J BRISMAR C.M. HESSER and G MATELL, Time courses of blood gas changes provoked by light and moderate exercise in man. *Acta physiol. scand.* 1964 *60* 1–17

BASON R., C.E. BILLINGS E.L. FOX and R. GERKE, Oxygen kinetics for constant work loads t arious altitudes. *J appl. Physiol.* 1973 *35* 497–500.

BEAVER, W.L. and K. WASSERMAN Transients in ventilation t start and end of exercise *J appl. Physiol.* 1968. *25* 390–399

BERG W E., Individual differences in respiratory gas exchange during recovery from moderate exercise. *Amer J Physiol.* 1947 *149* 597–610.

BEVEGÅRD B S. and J T SHEPHERD Regulation of the circulation during exercise in man. *Physiol Rev* 1967 *47* 178–213

BJURSTEDT H. and G WIGERTZ, Dynamics of arterial oxygen tension in response to sinusoidal work load in man *Acta physiol. scand.* 1971 *82* 236–249

BROMAN SVETLANA and G WIGERTZ, Transient dynamics of ventilation and heart rate with step changes in work load from diff rent load levels. *Acta physiol. scand.* 1971 *81* 54–74

CARLSSON A., G HANNAUER T CAREY and P.J HOLSBERG *Handbook of Analog Computation.* Electronic Associates, Inc., Princeton, New Jersey 1965 389 pp

CERRETELLI P R SIKAND and L.E. FARHI Readjustments in cardiac output and gas exchange during onset f exercise and recovery *J appl. Physiol.* 1966 *21* 1345–1350

CHAPMAN C.B (ed) *Physiology f Muscular Exercise* The American Heart Association Inc New York 1967 226 pp.

CHRISTENSEN E H and P HÖGBERG Steady-state O_2-deficit and O_2-debt t severe work *Arbeitsphysiologie* 1950 *14* 251 254

CLAUSEN J P and N.A. LASSEN Muscle blood flow during exercise in normal man studied by the [133]Xenon clearance method. *Cardiovasc Res.* 1971 *5* 245–254

CRAIG F N L.G CUMMINGS and W V BLEVINS Regulation of breathing at beginning of exercise *J appl. Physiol.* 1963 *18* 1183–1187

CUMMING G.R., Stroke volume during recovery from supine bicycle exercise *J appl. Physiol.* 1972. *32* 575–578

CUNNINGHAM D.J.C Regulation of breathing in exercise *Circulat Res* 1967 *20* 122–131

DAVIES C T.M., Cardiac frequency in relation to aerobic capacity for work. *Ergonomics* 1968. *11* 511–526

DEJOURS P., La régulation de la ventilation au cours de l'exercice musculaire chez l'homme. *J Physiol. (Paris)* 1959 *51* 163–261

DEJOURS, P The regulation of breathing during muscular exercise in man. A neuro-humoral theory In *The Regulation of Human Respiration*, edited by D.J.C Cunningham and B.B Lloyd. Blackwell Scientific Publications. Oxford 1963 535–547

DEJOURS P., Neurogenic factors in the control of ventilation during exercise. *Circulat Res.* 1967 *20* 146–153.

DEJOURS P R. FLANDROIS, R LEFRANÇOIS and A. TEILLAC, Etude de la régulation de la ventilation au cours de l'exercice musculaire chez l'homme *J Physiol. (Paris)* 1961 *53*. 321

DEJOURS P., J RAYNAUD C.L. CUENOD and Y LABROUSSE, Modifications instantanées de la ventilation au début et à l arrêt de l'exercice musculaire. Interprétation. *J Physiol. (Paris)* 1955 *47* 155–159

DRABKIN D.L. and J.H. AUSTIN Spectrophotometric studies. II Preparations from washed blood cells, nitric oxide hemoglobin and sulfhemoglobin. *J biol. Chem.* 1935 *112*. 51–65

DUBOIS, A.B., STELLA Y BOTELHO G.N BEDELL, R. MARSHALL and J.H COMROE Jr., A rapid plethysmographic method for measuring thoracic gas volume A comparison with a nitrogen washout method for measuring functional residual capacity in normal subjects. *J clin. Invest* 1956 *35* 322–326

EDWARDS R.H.T., *Circulatory Respiratory and Metabolic Changes at the Start of Exercise.* (PhD thesis) University of London. London. 1969 266 pp

EDWARDS R.H T., D.M. DENISON G. JONES C.T.M DAVIES and E.J.M. CAMPBELL Changes in mixed venous gas tensions at start of exercise in man. *J appl. Physiol.* 1972. *32*. 165–169

FAGRAEUS, L. and D LINNARSSON Autonomic origin of heart rate fluctuations at the onset of exercise *Report Lab Aviat Naval Med Karol. Inst., Stockholm.* April 1974 8 pp

FAGRAEUS L., J KARLSSON D LINNARSSON and B. SALTIN Oxygen uptake during maximal work at lowered and raised ambient air pressures. *Acta physiol. scand.* 1973 *87* 411–421

FARHI, L.E. and H. RAHN Gas stores of the body and the unsteady state. *J appl. Physiol.* 1955 *7* 472–484

FISHER R.A., *Statistical Methods for Research Workers.* 10th Ed Oliver & Boyd Ltd. Edinburgh. 1946 354 pp

FREYSCHUSS, ULLA Cardiovascular adjustment to somatomotor activation. The elicitation of increments in heart rate, aortic pressure and venomotor tone with the initiation of muscle contraction. *Acta physiol. scand.* 1970 Suppl. 342 63 pp

FUJIHARA Y J.R. HILDEBRANDT and J HILDEBRANDT Cardiorespiratory transients in exercising man I Tests of superposition. *J appl. Physiol.* 1973a *35* 58–67

FUJIHARA Y J HILDEBRANDT and J.R HILDEBRANDT Cardiorespiratory transients in exercising man II Linear Models. *J appl. Physiol.* 1973b *35*. 68–76

GAUER O.H. and H.L. THRON Postural changes in the circulation. In. *Handbook of Physiology Circulation*, edited by W.F Hamilton and P Dow Washington, D.C. Amer Physiol. Soc 1965 Sect. 2. Vol. III Chapt. 67 2409–2439

GELFAND R. and C.J LAMBERTSEN Dynamic respiratory response to abrupt change of inspired CO_2 at normal and high P_{O_2} *J appl. Physiol.* 1973 *35* 903–913

GEPPERT J and N ZUNTZ, Über die Regulation der Atmung. *Pflüg. Arch. ges. Physiol.* 1888 *42*. 189–245

GILBERT R. J.H AUCHINCLOSS Jr and G.H BAULE Metabolic and circulatory adjustments to unsteady-state exercise. *J appl. Physiol.* 1967 *22*. 905–912

GILBERT R., J.H AUCHINCLOSS Jr J BRODSKY and W BODEN Changes in tidal volume frequency and ventilation induced by their measurement. *J appl. Physiol.* 1972. *33* 252–254

GOLLNICK P.D., J KARLSSON KARIN PIEHL and B SALTIN Selective glycogen depletion in skeletal muscle fibres of man following sustained contractions. *J Physiol. (Lond.)* In press.

GRIMBY G., J BUNN and J MEAD Relative contribution of rib cage and abdomen to ventilation during exercise *J appl. Physiol.* 1968 *24* 159–166

GRIMBY G., N.J NILSSON and H. SANNE, Repeated serial determination of cardiac output during 30 min exercise. *J appl. Physiol.* 1966 *1* 1750–1756

GRODINS F.S., J.S. GRAY K.R. SCHROEDER, A I NORINS and R.W JONES Respiratory responses to CO_2 inhalation. A theoretical study of a nonlinear biological regulator *J appl. Physiol.* 1954 7 283–308

HANSEN E., Über die Sauerstoffschuld bei korperlicher Arbeit. *Arbeitsphysiologie* 1934 *8*, 151–171

HANSON J.S B.S. TABAKIN and E.J CALDWELL, Response of lung volumes and ventilation to posture change and upright exercise. *J appl. Physiol.* 1962, *17* 783–786

HARRISON W G J J.A. CALHOUN and T.R. HARRISON Afferent impulses as a cause of increased ventilation during muscular exercise. *Amer J Physiol* 1932, *100* 68–73

HENRY F.M and JANICE DE MOOR, Metabolic efficiency of exercise in relation to work load t constant speed. *J appl. Physiol.* 1950 .. 481–487

HENRY F.M and JANICE C. DE MOOR, Lactic and alactic oxygen consumption in moderate exercise of graded intensity *J appl. Physiol.* 1956 *8*, 608–614

HERMANSEN L. and INGER STENSVOLD Prod ction and removal of lactate during exercise in man *Act physiol. scand.* 1972, *86*, 191–201

HLASTALA, M.P B. WRANNE and C.J LENFANT Cyclical variations in FRC and other respiratory variables in resting man. *J appl. Physiol.* 1973 *34* 670–676

HUGHES R.L., MARIE CLODE, R.H T EDWARDS, T.J GOODWIN and N L. JONES Effect of inspired O_2 on cardiopulmonary and metabolic responses to exercise in man. *J appl. Physiol.* 1968 *24* 336–347

JONES W.B., R.N. FINCHUM R.O RUSSELL Jr and T.J REEVES Transient cardiac output response t multiple levels of supine ex rcise. *J appl. Physiol.* 1970 *28* 183–189

JORFELDT L., M tabolism f L(+) lactate in human skeletal muscle during exercise *Acta physiol. scand* 1970 *78* Suppl 338 67 pp

KARLSSON J *Lactat and phosphagen concentrations in working muscle of man. Act physiol. scand* 1971 *81* Suppl. 358 72 pp

KNUTTGEN H.G., Oxygen debt after submaximal physical exercise. *J appl Physiol.* 1970 29 651–657

KNUTTGEN H.G and B. SALTIN Oxyge ptake muscle high-energy phosphates, and lactate in exercise under acute hypoxic conditions in man. *Acta physiol scand.* 1973 *87* 368–376

KROGH, A and J LINDHARD Measurements f the blood flow through the lungs of man. *Skand. Arch. Physiol.* 1912. 7 100–125

KROGH A and J LINDHARD The regulation f respiration and circulation during the initial stages of muscular work. *J Physiol. (Lond)* 1913 *47* 112–136.

LINDBORG B O WIGERTZ and TH. ÖDMAN, A beat-to-beat heart-rate meter with linear analog output for muscular exercise studies. *Report Lab A at Naval Med. Karol. Inst., Stockholm,* Dec. 1969 23 pp.

LINNARSSON D nd B LINDBORG Breath-by-breath measurement f respiratory gas exchange using on-line nalog computation. *Scand. J clin. Lab Invest* 1974 In press.

LINNARSSON D J KARLSSON L. FAGRAEUS and B SALTIN Muscle *metabolites* and oxygen deficit with exercise in hypoxia and hyperoxia *J appl. Physiol.* 1974 *36*, 399–402.

MARGARIA, R., H T EDWARDS and D.B. DILL, The possible mechanisms of contracting and paying the oxygen debt and the role of lactic acid in muscular contraction. *Amer J Physiol.* 1933 *106* 689–715

MARGARIA R., F MANGILI F CUTTICA nd P CERRETELLI The kinetics of the oxygen consumption at the nset f muscular exercise in man. *Ergonomics* 1965 *8* 49–54

MARQUARDT D.W An algorithm for least squares estimation of nonlinear parameters. *SIAM J* 1963 2 431–441

MATELL, G Time-courses of changes in ventilation and arterial gas tensions in man induced by moderate exercise *Acta physiol. scand* 1963 *58*, Suppl 206 53 pp

MEAD J N PETERSON G GRIMBY and J MEAD Pulmonary ventilation measured from body surface movements. *Science* 1967 *156* 1383–1384

MILHORN H T J R. BENTON R ROSS and A C. GUYTON A mathematical model f the human respiratory control system *Biophys. J* 1965 5 27–46

DE MOOR JANICE C Individual differences in o ygen debt curves related t mechanical eff nd sex *J appl. Physiol.* 1954 6 460–466

PAULEV P.-E., Respiratory and cardiac responses to exercise in man. *J appl. Physiol.* 1971 *30* 165–172.

PERNOW B and B. SALTIN (editors) *Muscle Metabolism during Exercise*. Plenum Press. New York London 1971 558 pp

PETRO J K., A.P HOLLANDER and L.N BOUMAN Instantaneous cardiac acceleration in man induced by a voluntary muscle contraction. *J appl. Physiol* 1970. 29 794–798.

DI PRAMPERO P.E., C.T.M DAVIES, P CERRETELLI and R. MARGARIA, An analysis of O_2 debt contracted in submaximal exercise *J appl. Physiol.* 1970 *29* 547–551

DI PRAMPERO P.E., L. PEETERS and R MARGARIA, Alactic O_2 debt and lactic acid production after exhausting exercise in man. *J appl. Physiol.* 1973 *34* 628–632

RAYNAUD J H BERNAL, J.P BOURDARIAS P DAVID and J DURAND Oxygen delivery and oxygen return to the lungs at onset of exercise in man. *J appl. Physiol.* 1973 *35* 259–262.

REYNOLDS W.J and H T MILHORN Jr., Transient ventilatory response to hypoxia with and without controlled alveolar PCO_2 *J appl. Physiol.* 1973 *35* 187–196

ROBINSON S Experimental studies of physical fitness in relation to age *Arbeitsphysiologie* 1938 *10* 251–323

ROCHESTER, D.F J DURAND J.O PARKER, H W FRITTS Jr and R.M. HARVEY Estimation of right ventricular output in man using radioactive krypton (Kr^{85}). *J clin. Invest.* 1961 *40* 643–648.

ROSENHAMER, G Estimation of alveolar PO_2 *Acta physiol scand.* 1972 *84* 22–28.

ROWELL, L.B Human cardiovascular adjustments to exercise and thermal stress. *Physiol. Rev* 1974 *54* 75–159

SALTIN B P.D GOLLNICK, B.O ERIKSSON and KARIN PIEHL, Metabolic and circulatory adjustments at onset of maximal work. *Symposium on the Onset of Exercise* edited by A Gilbert and Patricia Guille University of Toulouse. 1971 63–76

SCHOLANDER P.F., Analyzer for accurate estimation of respiratory gases in one-half cubic centimeter samples. *J biol. Chem.* 1947 *167* 235–250

SJÖSTRAND T., Exercise tests. In *Clinical Physiology* edited by T Sjöstrand. Svenska Bokförlaget. Stockholm 1967 515–530

STEGEMANN J Beitrag zu Dynamik der Atmungsregelung bei leichter Muskelarbeit. *Z. Biol.* 1958. *110* 449–456

STEGEMANN J., Zum Mechanismus der Pulsfrequenzeinstellung durch den Stoffwechsel. IV Zur Frage der Lokalisation der stoffwechselempfindlichen Muskelreceptoren. *Pflüg. Arch. ges. Physiol.* 1963 *276* 511–524

STEGEMANN J and TH KENNER, A theory on heart rate control by muscular metabolic receptors. *Arch. Kreisl.-Forsch.* 1971 *64* 185–214

STRÖM G The influence of anoxia in lactate utilization in man after prolonged muscular work. *Acta physiol. scand.* 1949 *17* 440–451

WADE, O L. and J.M BISHOP *Cardiac Output and Regional Blood Flow* Blackwell Scientific Publications, Oxford 1962 268 pp

WASSERMAN K., A.L. VAN KESSEL and G.G BURTON Interaction of physiological mechanisms during exercise *J appl. Physiol.* 1967 *22* 71–85

WHIPP B.J Rate constant for the kinetics of oxygen uptake during light exercise. *J appl. Physiol.* 1971 *30* 261–263

WHIPP B.J and K. WASSERMAN Efficiency f muscular work *J appl. Physiol* 1969 *26*. 644–648

WHIPP B.J and K. WASSERMAN Oxygen uptake kinetics fo various intensities of constant-load work. *J appl. Physiol.* 1972 *33* 351–356

WHIPP B.J C. SEARD and K. WASSERMAN Oxygen deficit-oxygen debt relationships and efficiency of anaerobic work. *J appl Physiol* 1970 *28* 452–456

WIGERTZ O A low-resistance flow meter for wide-range ventilatory measurement. *Respir Physiol.* 1969 7 263–270

WIGERTZ, O Dynamics of ventilation and heart rate in response to sinusoidal work load in man. *J appl. Physiol.* 1970 *29* 208–218

WIGERTZ, O., Dynamics of respiratory and circulatory adaptation to muscular xercise in man. A systems analysis approach. *Acta physiol. scand* 1971 *82*. Suppl. 363 32 pp

ISBN 91–7222–082–1
Sandt Offset Stockholm 74

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 416

AFTERHYPERPOLARIZATION AND THE CONTROL OF REPETITIVE FIRING IN SPINAL NEURONES OF THE CAT

BY

BENGT GUSTAFSSON

GÖTEBORG 1974

I Afterhyperpolarization properties

A AHP conductance time course

1 α-motoneurones The AHP recorded in α-motoneurones lasts 40 200 ms and reaches a peak value 10-20 ms after the onset of the action potential (Brock et al 1952 Eccles et al 1958 Kuno 1959) Usually the rapid falling phase of the spike is not immediately followed by the AHP but is succeeded by a phase of slow repolarization lasting for some ms the delayed depolarization (DD; Kernell 1964 Nelson and Burke 1967) In some motoneurones a clear depolarizing potential can be seen in this phase giving a "hump-type-DD"

From the effect of membrane potential displacements and of iontophoresis on the AHP voltage (Coombs Eccles and Fatt 1955) it was concluded that the AHP in motoneurones is mainly resulting from a membrane conductance increase to potassium ions The time course of the underlying conductance process was however not deduced It has thus not been clear to which extent the long time-to-peak AHP was given by the conductance process as suggested by Eccles (1957) or by the process giving rise to the DD (Kernell and Sjöholm 1972) The origin of this latter process is also unknown but it has been suggested to be caused by current fed back from the spike invasion into the dendrites (Granit et al 1963 Kernell 1964 Nelson and Burke 1967) or by an unspecific conductance change (mainly sodium; Kernell and Sjöholm 1972)

In Paper I the conductance time course during the AHP was determined by the application of short current pulses injected through the recording microelectrode. The time course so measured, displayed an initial fast decay during the rapid hyperpolarizing phase of the AHP followed by a phase of constant or slightly increasing (or decreasing) conductance lasting to the peak of the AHP Thereafter the conductance decreased in a rather exponenti[illegible] during the [illegible] phase of the AHP A similar conducta[illegible] course was [illegible] from the AHP voltage, under the assu[illegible] the AHP [illegible] given by a K conductance increase The c[illegible] from the experimental ones, by showing [illegible] dip in [illegible] decay phase This dip [illegible] to [illegible] with the [illegible] was also absent [illegible] DD [illegible]

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 416

AFTERHYPERPOLARIZATION AND THE CONTROL OF REPETITIVE FIRING IN SPINAL NEURONES OF THE CAT

BY

BENGT GUSTAFSSON

GÖTEBORG 1974

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 416

FROM THE DEPARTMENT OF PHYSIOLOGY UNIVERSITY OF GÖTEBORG, SWEDEN

AFTERHYPERPOLARIZATION AND THE CONTROL OF REPETITIVE FIRING IN SPINAL NEURONES OF THE CAT

BY

BENGT GUSTAFSSON

GÖTEBORG 1974

This thesis mainly constitutes a summary of the following articles

I Baldissera, F and B Gustafsson, Afterhyperpolarization con ductance time course in lumbar motoneurones of the cat. Acta physiol scand 1974 In press

II Baldissera, F and B Gustafsson, Firing behaviour of a neurone model based on the afterhyperpolarization conductance time course First interval firing Acta physiol scand 1974 In press

III Baldissera, F and B Gustafsson Firing behaviour of a neurone model based on the afterhyperpolarization conductance time course and algebraical summation. Adaptation and steady state firing Acta physiol scand. 1974 In press

IV Gustafsson, B S Lindström and M Takata A re-evaluation of the afterhyperpolarization mechanism in dorsal spinocerebellar tract neurons Brain Res 1971 35 543-546

V Gustafsson, B S Lindström and M Takata Repetitive firing in dorsal spinocerebellar tract neurones Brain Res 1972 47 506-509

VI Gustafsson B and P Zangger Depression of the afterhyperpolarization in dorsal spinocerebellar tract neurones Brain Res 1974 72 320-323

These papers will be referred to by their Roman numericals in the text.

Göteborgs Grafiska Grupp AB

CONTENTS

INTRODUCTION 5

AIM OF THE PRESENT STUDY 7

RESULTS AND COMMENTS 8

I Afterhyperpolarization properties 8

A AHP conductance time course 8
1 α-motoneurones 8
2 DSCT neurones 9

B AHP summation 12

II Repetitive firing of a neurone model based on the AHP conductance time course compared to that of real neurones 13

A First interspike interval computation 15
1 Computation with conductance plateau 15
2 Computation without a conductance plateau 18

B Computed adaptation in the neurone model 21
1 Computation with conductance plateau and algebraical summation 22
2 Computation without conductance plateau and with algebraical summation 22
3 Computation without conductance plateau and with AHP depression 24

C Computed steady state firing 27

III Model limitations 28

GENERAL DISCUSSION 31

A AHP and firing control 31

B Model considerations 34

C Descriptive terminology 35

D Other CNS neurones 36

E Functional implications 37

SUMMARY AND CONCLUSIONS 40

ACKNOWLEDGEMENTS 43

REFERENCES 44

INTRODUCTION

In the nervous system information carried between nerve cells is encoded in the frequency of action potentials transmitted along the axons The steady excitation built up by the continuous synaptic activity impinging upon a neurone is thus converted into a discontinuous output i e a train of action potentials whose frequency is related to the amount of excitation The encoding procedure, i e the mechanism controlling the relation between excitation and frequency was early visualized by Adrian (Adrian and Zotterman 1926) as follows After the neurone (or receptor) has fired an action potential, the neurone is in a refractory state, i e the threshold for giving an action potential is increased The threshold increase is then slowly decaying with a certain time course With a just suprathreshold stimulation the interval between the impulses will then be equal to the duration of the refractoriness Increasing the excitation the refractoriness will be overcome at an earlier interval i e the frequency of action potentials will increase There will thus be a direct correspondence between the amount of excitation and the frequency of action potentials and this relation will be completely determined by the size and shape of the time course of the refractoriness When studied with intracellular technique the nerve cells were in fact, found to possess a longlasting refractoriness following each action potential In α-motoneurones in the cat spinal cord the action potential was followed by an afterhyperpolarization (AHP) lasting 40–200 ms (Brock Coombs and Eccles 1952 Eccles Eccles and Lundberg 1958 Kuno 1959)

The hypothesis of Adrian could then be tested in these neurones by comparing the time course of the refractoriness given by the AHP with the relation between excitatory current and impulse frequency as outlined above The technique used consists in injecting the excitatory current as constant current pulses from an intracellular microelectrode and measuring the discharge frequency at various current strengths (Granit, Kernell and Smith 1963 Granit, Kernell and Shortess 1963 a, b) In such investigations (Kernell 1965 b c d) it was demonstrated that the minimal rhythmic frequency was correlated with the duration of the AHP as predicted by Adrian, and also other correlations between AHP parameters and the firing were found. However due to the lack of sufficient knowledge of the mechanism underlying the AHP in motoneurones more critical tests aimed at determining to what extent the firing was regulated by the AHP in the manner described by Adrian could not be made and certain findings were even indicating that other mechanisms could be of importance (Kernell 1965 c Granit, Kernell and Lamarre 1966 b) It was understood that if the encoding should be

only determined by the AHP time course, the afterpotential mechanism must be invariant of all other activity in the cell If instead the amplitude of the afterpotential for example was a function of the degree of the antidromic invasion of the nerve impulse into the dendritic tree and if this invasion could be affected by various factors then these factors could also be of considerable importance for the firing regulation Even if this view still pointed to the importance of refractoriness for firing regulation it precluded the possibility to predict firing behaviour from the shape of the refractoriness as measured after single action potentials This alternative hypothesis originated in the finding that in many motoneurones the frequency–current (f/I) relation at higher frequencies suddenly bent upwards and displayed a second linear range (secondary range) whose characteristics could be modified by synaptic activity and whose appearance was correlated with a sudden change in the interspike voltage course implying a change in the AHP properties (Kernell 1965 c Granit et al 1966 b) This hypothesis was later expanded by Schwindt and Calvin (1972) who suggested that more or less continuous changes in *the spatial distribution of the AHP could be a factor of importance for* the input–output relation in motoneurones

There are, however some evidence that the above alternative hypothesis is disputable Recently Kernell and Sjöholm (1973) demonstrated that by modifying voltage clamp equations from the myelinated frog nerve fibre to give a longlasting AHP some of the features of the firing behaviour of motoneurones previously indicative of a spatial factor could be simulated However even if this model rather well simulated the f/I relations in motoneurones it did not well simulate the characteristic modifications of the interspike voltage trajectories with increasing current described for real motoneurones (Schwindt and Calvin 1972, Schwindt 1973) Moreover it is not clear to which extent the mechanism underlying the AHP in this model is similar to that in motoneurones

The α-motoneurones have in many respects been used as a model neurone for central neurones The firing behaviour of the few other neurones investigated was however found to differ from that of α-motoneurones to some extent (Kandel and Spencer 1961 Kuno and Miyahara 1968 Eide, Fedina, Jansen Lundberg and Vyklický 1969 Koike, Mano Okada and Oshima 1970 Baker and Precht 1972) For example in the cells belonging to the dorsal spinocerebellar tract (DSCT) the f/I relation is linear throughout its frequency range without displaying a 'secondary range as in motoneurones and has a much steeper slope than in motoneurones (Eide et al 1969)

Two different suggestions have been forwarded to explain this difference According to one of these, the absence of a secondary range could be accounted for by a lack of change in dendritic invasion

with increasing current, thus implicating the importance of spatial factors in firing regulation (Eide et al 1969) The other hypothesis pointed instead to the possibility that the AHP mechanism in DSCT neurones is of a different nature than that of motoneurones a suggestion which has also received some experimental support (Eide et al 1969 Kuno Miyahara and Weakly 1970)

AIM OF THE PRESENT STUDY

Even if it is generally agreed that the AHP is a factor of considerable importance for the firing regulation in central neurones the exact role of it and of other factors are still uncertain or as recently drastically stated (Schwindt and Calvin 1973 b) 'the mechanisms underlying rhythmic firing in motoneurones are virtually unknown a statement then referable to other central neurones as well. This statement is in one sense justified as long as no direct correlation between the AHP properties and the firing behaviour is made, outlining the role of the AHP and the necessity and possible role of other factors

The aim of the present study is to demonstrate to which extent the AHP can account for the firing behaviour in two different types of spinal neurones the α-motoneurones and the DSCT neurones The study will cover two sections The first section will deal with investigations on the AHP mechanism in motoneurones and DSCT neurones in order to deduce the nature and the time course of the underlying processes In the second section the firing behaviour predicted by a model based on these processes will be computed and compared to the firing behaviour of the real neurones

RESULTS AND COMMENTS

I Afterhyperpolarization properties

A AHP conductance time course

1 α-motoneurones The AHP recorded in α-motoneurones lasts 40 200 ms and reaches a peak value 10-20 ms after the onset of the action potential (Brock et al 1952 Eccles et al. 1958 Kuno 1959) Usually the rapid falling phase of the spike is not immediately followed by the AHP but is succeeded by a phase of slow repolarization lasting for some ms the delayed depolarization (DD; Kernell 1964 Nelson and Burke 1967) In some motoneurones a clear depolarizing potential can be seen in this phase giving a "hump-type-DD"

From the effect of membrane potential displacements and of iontophoresis on the AHP voltage (Coombs Eccles and Fatt 1955) it was concluded that the AHP in motoneurones is mainly resulting from a membrane conductance increase to potassium ions The time course of the underlying conductance process was however not deduced It has thus not been clear to which extent the long time-to-peak AHP was given by the conductance process as suggested by Eccles (1957) or by the process giving rise to the DD (Kernell and Sjöholm 1972) The origin of this latter process is also unknown, but it has been suggested to be caused by current fed back from the spike invasion into the dendrites (Granit et al 1963 Kernell 1964, Nelson and Burke 1967) or by an unspecific conductance change (mainly sodium; Kernell and Sjöholm 1972)

In Paper I the conductance time course during the AHP was determined by the application of short current pulses injected through the recording microelectrode The time course so measured, displayed an initial fast decay during the rapid hyperpolarizing phase of the AHP followed by a phase of constant or slightly increasing (or decreasing) conductance lasting to the peak of the AHP Thereafter the conductance decreased in a rather exponential manner during the repolarizing phase of the AHP A similar conductance time course was also computed from the AHP voltage, under the assumption that the AHP was only given by a K conductance increase The computed time courses differed, from the experimental ones by showing a shortlasting dip in the fast initial decay phase This dip was found to be correlated with the size of the DD and was also absent in neurones lacking an apparent DD It was concluded

that the AHP in motoneurones was given by a conductance time course *as suggested by Eccles (1957)* and that the DD was given by a short-lasting current, not giving rise to the long time-to-peak AHP

It was assumed by Coombs et al (1955) that the mechanism underlying the AHP was not much affected by the membrane potential. This assumption was largely corroborated in the present study by showing a constancy both in the voltage drop caused by a current pulse evoked during the AHP at *different membrane potential levels and in the computed* conductance time course at different potential levels This conclusion was of considerable significance since it meant that the AHP potassium conductance could be described only as a function of time and not as a function of both voltage and time It was concluded that the AHP in α-motoneurones was given by a complex function in time describing an initial fast decay a plateau region lasting to the peak of the AHP and a slow exponential decay A mathematical function describing this time course was then derived.

2 DSCT neurones Previous investigations (Kuno and Miyahara 1968 Eide et al 1969 Kuno et al. 1970) had shown the AHP in DSCT neurones to be appreciably smaller and shorter than that in motoneurones As stated in the Introduction these investigations had also given evidence that the AHP in DSCT neurones was not caused by a K conductance mechanism as in α-motoneurones Kuno et al. (1970) had found that there was no conductance increase during the AHP that the AHP voltage was not potential dependent, that the AHP polarized the membrane to more negative levels than the potassium equilibrium potential and that the AHP had a temperature dependence incompatible with a passive ionic process They concluded that the AHP instead was caused by the activation of a sodium electrogenic pump On the other hand, Eide et al. (1969) found the AHP voltage to be very sensitive to polarizing currents but found only small, if any conductance changes They suggested that the AHP at least partly could be caused by a sodium conductance decrease The apparent discrepancies in the reported results made a complete reinvestigation of the AHP properties in DSCT neurones necessary

In such a reinvestigation of the AHP properties in DSCT neurones (Paper IV) it was demonstrated that the AHP in these cells as in motoneurones is caused by a conductance process for potassium ions By displacement of the membrane potential with current injections the AHP voltage was found to increase with depolarization and decrease by hyperpolarization, as found by Eide et al (1969) and as expected for a conductance process The conductance during the AHP was measured by application of current pulses and a clear conductance increase was found in all the cells tested By applying the same pulses during another

hyperpolarizing pulse, it was established that the measured conductance changes were not secondary to the hyperpolarization itself These find ings indicated that the AHP was neither given by an electrogenic pump nor by a sodium conductance decrease process However if the pump or the sodium conductance were potential dependent, increasing with depolarization and decreasing with hyperpolarization similar experimental results could, in fact, also be produced by these mechanisms In a few neurones the AHP could, however be reversed and current pulses applied during these reversed AHPs demonstrated a conductance increase (Gustafsson Lindström and Takata, to be published) Since these experimental results could not be given by the two former mechanisms it can be concluded that the AHP in DSCT neurones is given by a K conductance mechanism

It had further been argued by Kuno et al (1970) that the AHP could not be given by a K conductance process since the AHP polarized the membrane to more negative levels than the K equilibrium potential and the AHP decay had a temperature dependence incompatible with a passive ionic process The last objection was not relevant since the temperature dependence of the decay of passive ionic processes in contrast to that of the amplitude, is rather similar to that of active metabolic processes (Hodgkin and Huxley 1952) This point is made very clear by the recent finding that there is a temperature dependence of the AHP decay also in motoneurones (Pierau, Klee and Faber 1971) The objection that the AHP polarized the membrane to more negative values than the potassium equilibrium potential was also easily ruled out since Kuno et al (1970) had not estimated any potassium equilibrium potential In nerve cells the potassium equilibrium potential has only been estimated from the reversal potential of the AHP Since Kuno et al did not find any potential dependence of the AHP no reversal potential and thus K equilibrium potential could be estimated The equilibrium potential was instead taken as the "reversal potential of the initial overswing immediately following the falling phase of the spike However due to the presence of a delayed depolarization in this region, this "reversal potential is not equal to the K equilibrium potential

Earlier investigations (Kuno and Miyahara 1968 Eide et al. 1969 Kuno et al. 1970) had found the AHP in DSCT neurones to be smaller and shorter than that of motoneurones In the present study the computed time course of the conductance underlying the AHP in DSCT neurones was also found to be different from that in motoneurones, displaying no or only a small plateau phase before the subsequent exponential decay (see Fig 1) This time course was also reflected in a considerable shorter time-to-peak AHP being only on the average 7 0 ms in DSCT neurones (Gustafsson, Lindström and Takata, to be published) compared to 10-20 ms in motoneurones Otherwise the computed $G_K(t)$ had a similar

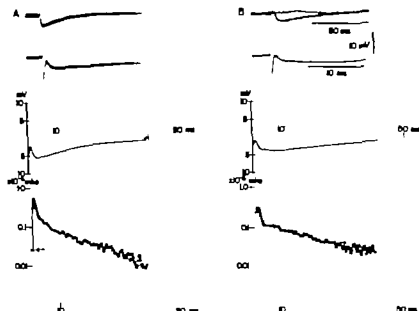


Fig 1 Computed AHP conductance time course from two DSCT neurones The conductance time courses computed from the AHP voltage are shown for two DSCT neurones with a short time–to–peak AHP (A) and a long time–to–peak AHP (B) Records from which the measurements were taken are shown above each graph Observe the small plateau in the computed capacitance in B corresponding to the slow hyperpolarizing phase of the AHP The initial dip given by the DD is marked in A with an arrow Input resistance (MΩ) membrane capacitance (nF 10^{-9} F) are 2 20 2 85 (A) 2 90 3 60 (B) AHP equilibrium potential for both cells assumed to be 20 mV below resting level

course to that in motoneurones i e there was an initial dip corresponding to the DD followed by an initial fast decay during the hyperpolarizing phase of the AHP and a slow exponential decay during the repolarizing phase of the AHP A similar conductance time course was also found in one DSCT neurone with the use of short constant current pulses (Gustafsson Lindström and Takata, to be published)

B AHP summation

In some central neurones including the α-motoneurones and the DSCT neurones the AHP following a short train of spikes is larger than that following a single spike i e the successive AHPs summate (Ito and Oshima 1962 Eide et al 1969 Baker and Precht 1972 Kitai, Tanaka Tsukahara and Yu 1972) Since this summation has been considered as a mechanism for the decrease in frequency (adaptation) occurring in neurones after the onset of a constant current pulse (Kernell 1968 1972 Calvin and Schwindt 1972) knowledge of its properties should be of importance for the understanding of firing regulation This summation effect is largest at short interspike intervals and decays to zero when the interspike interval approaches the duration of the AHP In α-motoneurones the time course of the summation of the AHP voltage between two successive AHPs was not unlike the time course of the AHP itself (Calvin and Schwindt 1972) In the present investigation the conductance summation between two successive AHPs was studied (Paper III) and a similar conclusion was reached. The increase in the AHP peak conductance (computed and estimated with short current pulses) of the second AHP was following a course rather similar to that of the conductance of the first AHP i. e there seemed to be an algebraical summation between the two AHPs There was however in most of the cells studied a quantitative deviation from the algebraical summation, the absolute summation being both larger and smaller

In DSCT neurones the AHP interaction had previously only been studied with short interspike intervals between the action potentials and an AHP summation had been found (Eide et al. 1969 Kuno et al 1970) At longer interspike intervals the AHP interaction was however more complex than in motoneurones (Paper VI) In the majority of the cells the AHP following two spikes was in fact, smaller than that following a single spike the depression being maximal at an interval corresponding to the AHP duration The falling phase and the initial hyperpolarizing overswing of the spike was unaffected by the conditioning spike demonstrating a separation of the K conductance underlying the AHP and that giving the falling phase of the action potential The AHP voltage from the peak of the DD was symmetrically decreased and subsequent conductance computation demonstrated the depression to symmetrically affect the whole computed time course as described earlier i e both the first fast decay and the subsequent slow decay (Gustafsson and Zangger to be published) The finding that the neurones with large depression also had a smaller AHP summation at short intervals suggested that the depression and summation, in fact, were both present throughout the interspike interval An interesting observation was here that, in the neurones with large depression the hyperpolarizing phase of the AHP following a single spike was faster than that

following two spikes in quick succession in spite of an AHP summation This difference in AHP time course can however be explained as a consequence of the depression, being caused by the linear summation of the conductance remaining from the first spike to the depressed conductance from the second spike (Gustafsson and Zangger to be published)

The depressive effect was not accumulating the successive AHPs in a long train (with an interval = AHP duration) being of the same size as the second one No apparent increase in depression was found when conditioning a single spike with a train of spikes instead of with a single spike (Gustafsson and Zangger to be published)

Otherwise the AHP interaction in DSCT neurones behaved very similar to that given by a linear summation The final value of the AHP summation was reached in a rather exponential fashion, the slope and peak value increasing with decreasing interval There was no change in the time constant of AHP decay (computed time course) between AHPs following a single spike and a train of 15 spikes (Gustafsson and Zangger to be published)

II Repetitive firing of a neurone model based on the AHP conductance time course compared to that of real neurones

The aim of the present investigations was to study to which extent the firing behaviour of motoneurones and DSCT neurones is accounted for by the AHP properties From the results presented in the preceding section and with some simplifying assumptions of the neuronal properties a neurone model for repetitive firing based on the AHP properties can be formulated as follows

1 The K conductance underlying the AHP is potential independent and has a time course as described in the preceding section (see Paper I and Fig 2) Its equilibrium potential is constant

2 The K conductance evoked by one spike is unaffected by the succeeding spikes

3 The K conductance evoked by the second and following spikes after the onset of a constant current can be multiplied with a correction factor in respect to that evoked by the first spike

4 The delayed depolarization is given by a shortlasting depolarizing current pulse, which is constant for each spike (see Fig 2)

5 *All other conductances but the K conductance are constant* throughout the interspike interval and their equilibrium potentials are constant

6 The threshold for spike activation is constant, the spike duration is constant and the falling phase of the spike ends at a constant voltage level

7 The complex geometry of the neurone can be neglected and the neurone can be represented as a sphere

Before entering the computed and experimental firing behaviour demonstrated in the present investigation previous results on the repetitive firing in motoneurones and DSCT neurones and the descriptive terminology developed in these investigations will be reviewed

In most α-motoneurones and DSCT neurones the application of a constant current pulse gives rise to a repetitive discharge, whose frequency is dependent upon the current strength After the onset of the current pulse there is a rapid decrease in frequency (adaptation) which is usually completed within the first few spikes but may continue for a longer time (Kernell 1965 b Eide et al 1969)

In α-motoneurones the frequency-current (f/I) relation and interspike voltage trajectory behaviour has mostly been studied after adaptation is completed, i e at the steady state The steady state f/I relation is composed of three linear segments, the 'primary secondary and 'tertiary range (Kernell 1965 c Schwindt 1973) The 'primary range extends from the minimal rhythmic frequency up to a maximal frequency corresponding to 1/time-to-peak AHP followed, in some motoneurones by a second, linear steeper segment, the 'secondary range (Kernell 1965 c) In other motoneurones the continuous firing failed before reaching higher frequencies and only a 'primary range was found A third linear segment, the 'tertiary range, with a slope usually less than that in the secondary range, has been found in some motoneurones (Schwindt 1973)

In the trajectory analysis (Schwindt and Calvin 1972 Schwindt 1973) the AHP voltage was subdivided in two segments the scoop being the descending and bottoming portion of the AHP and the ramp being the subsequent repolarization to firing level. It was suggested that the different firing ranges were associated with a special trajectory behaviour In the 'primary range the trajectory was characterized by a scoop-change behaviour i e with increasing current the size of the scoop was diminished while the ramp slope was constant (or stereotyped) In the 'secondary range the scoop had an upward convex shape instead of the upward concave shape found in the 'primary range The firing was in the secondary range controlled by a ramp change behaviour i e the

firing level was now reached by changing the slope of the trajectory The entrance into the "tertiary" range was associated with a sudden change in trajectory shape and the interspike trajectory assumed an angular form with a downward peak (Schwindt 1973) Within the "tertiary" range there was again a scoop-change behaviour i e with increasing current the peak was decreased while the slope of the ramp remained constant A similar f/i and trajectory behaviour was also described for the first interspike intervals i e before adaptation

In the DSCT neurones the steady state f/i relation was found to exhibit only one linear segment, reaching up to very high frequencies (Eide et al 1969) For the first interspike intervals i e before adaptation, only the higher frequency range was investigated, displaying an upward concave relation (Kuno and Miyahara 1968) No analysis of the trajectories was done in these studies

The computation of the firing behaviour of the neurone model was first performed without the assumption 2, i e for a model without AHP "*summation*" *This would then be similar to the first interspike* interval after the current onset in real neurones

A First interspike interval computation

1 Computation with conductance plateau. With this "motoneurone-like" model most of the features already described from *motoneurones could be found* (Paper II) With *increasing current the* upward concave scoop first flattened and then assumed an upward convex shape At very high currents the trajectory assumed the downward angular shape described for real neurones The f/i relation also showed three more or less distinct segments Below the frequency corresponding to 1/time-to-peak AHP the f/i slope started to increase considerably i.e the f/i relation was displaying a secondary range. At still higher currents the f/i slope decreased again and assumed an upward convex shape There were, however some clear differences between the model behaviour and the behaviour of real neurones as described earlier In the model, the ramp slope was not linear at the very low frequencies but displayed a slightly upward convex shape The approximated ramp slope was not constant but increased with increasing current. However by scrutiny of the records published by Schwindt and Calvin (1972) it was clear that the model behaviour was not in contrast to their results While Schwindt and Calvin emphasized the linear and the stereotyped character of the ramp the real motoneurone trajectories were in fact also upward convex in shape at the very low frequencies and the ramp slope at increasing current could, in fact, also be "force fitted" by steeper lines in much the same way as in the model (see also Paper III)

The flattening and the subsequent "reversal" of the scoop while gradual, was earlier described (Schwindt and Calvin 1972) as taking place in connexion with the entrance into the 'secondary range of firing In the model this was not the case; the scoop change occurring already well within the 'primary range, the exact timing depending upon the shape of the $G_K(t)$ plateau.

Since the previous studies were mostly concerned with the steady state firing the relation between the first interval trajectory modifications and the f/I shape was investigated and the behaviour displayed by the model was in fact also found in the motoneurones examined

It was earlier described that a hump-type delayed depolarization seemed to appear in some motoneurones in connexion with the entrance into the 'secondary range (Schwindt and Calvin 1972) The published records indicated, however that this "DD' was appreciably later than the DD found at resting level and suggested to us that it instead was coupled with the $G_K(t)$ local minimum (see Paper I) This later hump should then be preceded in real motoneurones by a DD and such a double-hump trajectory sequence was also found in real motoneurones

The rise to firing level during the 'primary range was earlier described as being stereotyped (Schwindt and Calvin 1972) while during secondary range the trajectory slope was increasing with increasing current (Schwindt 1973) Also in the model the rate of rise to firing level increases with increasing current during the secondary range more than during the 'primary range of firing This trajectory behaviour during secondary range is however not related to entering the secondary range but is a continuation of the scoop rotation around the pivot point given by the falling phase of the spike present also all through the 'primary range

The entrance into the 'tertiary range of firing was earlier described (Schwindt 1973) to occur together with a sudden change in trajectory shape, indicative of a sudden change in cell properties This sudden change is also reproduced by the model and is occurring when the scoop is fully rotated and thus the $G_K(t)$ plateau region passed The trajectory will then assume the simple downward angular shape in the model as in real neurones (Schwindt 1973)

The model behaviour gave also an additional prediction of firing behaviour When computing the first interval firing with a pronounced $G_K(t)$ local minimum no interval values corresponding to the region surrounding the local maximum were found, i e there was a discontinuity in the f/I relation, not earlier described for motoneurones Such a jump in the first interval f/I relation was however also displayed by some of the motoneurones examined, thus giving a discontinu

ity between the upper part of the 'primary' range and the lower end of the upper convex part of the f/i relation or 'tertiary' range

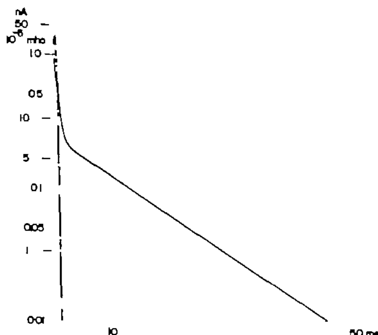


Fig 2 Graphic representation of the equations representing the AHP K conductance and DD current in DSCT neurones Dotted line is representing the DD current and is given by the equation 60 x EXP $(-t^{.5})$ x t^{6} expressed in nA The continuous line is representing the AHP K conductance and is given by the equation 0 25 x EXP (-t/14) + 1 1 x EXP $(-t^{1\ 2})$ expressed in 10^{-6} mho

2 Computation without a conductance plateau
The trajectories resulting from this "DSCT" like model could also be subdivided into a scoop and a ramp segment (see Fig 3) The shape of these trajectories and their modification with increasing current injection was however somewhat different from that of the "motoneurone" ("Mn") model Moreover the trajectory shape was also much dependent upon the size of the DD (or the magnitude of the initial fast $G_K(t)$ decay) With a DD present (Fig 3 A) the scoop started at the peak of the DD and reached an early peak, followed by an almost linear slightly upward convex ramp With increasing current the scoop decreased successively while the ramp slope increased This pattern of trajectory modification continued up to very short interspike intervals (note the upward concavity still at 11 5 nA in Fig 3 A) until the firing started at the peak of the DD and the trajectory assumed a downward angular shape. At all current strengths the DD gave an

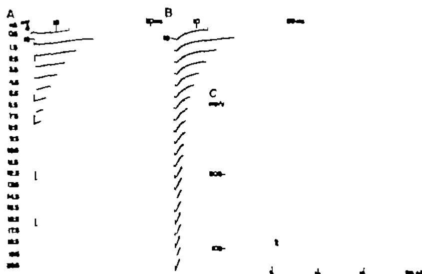


Fig 3 DSCT model firing The interspike voltage trajectories (A and B) and the corresponding f/I relations (C) computed with the aid of the equations in Fig 2 and the differential equation in Paper II. In A and filled circles in C are shown the computations performed with a DD In B and open circles in C without DD The initial conditions (see Paper II) were 0 5 ms and 10 mV Model input resistance is 1 0 MΩ capacitance 3 0 nF and K equilibrium potential 30 mV below threshold

initial upward convexity not to be confused with the upward convexity given by the $G_K(t)$ plateau, lacking in this model version The f/I relation given by these trajectories (filled circles in C) was also somewhat different from that of the 'Mn model Even if the upward concave-convex (or sigmoid) relation could easily be subdivided into three segments roughly corresponding to a primary a secondary and a tertiary range, these ranges were continuously connected and no jump or firing acceleration as that in the 'Mn model were present (Paper II) The transition between the various firing ranges was also taking place without any special trajectory event, i e there was no special primary 'secondary or 'tertiary range trajectory behaviour (compare Fig 3 A and filled circles Fig 3 C)

In the trajectories computed without a DD (see Fig 3 B) the scoop was given by the brief hyperpolarizing overswing directly followed by a ramp displaying a considerable upward convex curvature With increasing current, this ramp given by the exponential $G_K(t)$ decay rotated (in

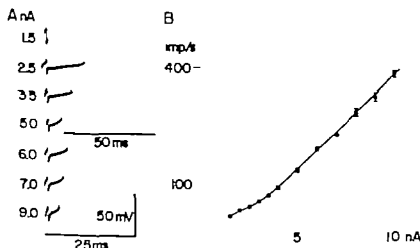


Fig 4 Trajectory shape and first interval f/I relation for a DSCT neurone In the f/I relation in B is given for each current strength the average value (filled circles) ± S D (n = 4–6) (At some current strengths the ± S D is within the area of the filled circles) Observe that the transition between the lower segment of the f/I relation and the upper steeper segment occurs without any change in trajectory shape Compare the trajectories in A with those in Fig. 3 A

much the same way as the scoop given by the $G_K(t)$ plateau in the "Mn" model) around the pivot point given by the falling phase of the spike The corresponding f/I relation (Fig 3 C open circles) did, however display the same sigmoid shape as with a DD (filled circles) but in a much more compressed form. Also with this model version there was no direct correlation between trajectory shape and f/I shape (compare Fig 3 B and 3 C open circles)

In previous investigations (Kuno and Miyahara 1968) the first interspike interval f/I relation in DSCT neurones was only studied in the higher frequency range, where an upward convex relation, as that demonstrated by the model was found The trajectory records given displayed the same upward convex–concave–linear shape as given by the DD–scoop–ramp sequence in the computations in Fig 3 A In the present study the first interval f/I relation was examined in 11 DSCT neurones over a much larger frequency range especially at the lower frequencies and the same sigmoid shape as found in Fig 3, was present in all these neurones (Paper V) Due to the invariable presence of a DD both the f/I relations and the trajectories corresponded better to the model version in A than in B The first interval trajectories and the corresponding f/I relation from a typical DSCT neurone is shown in Fig 4 ('tertiary range not illustrated) Observe in Fig 4 A that the trajectory modifications with increasing current are very similar in this neurone to that of the model in Fig 3 As in the model no correlation between the trajectory behaviour and f/I shape was found In none of these 11 cells was there any sudden jump or discontinuity in the f/I relation or a sudden change in trajectory shape The small $G_K(t)$ plateau probably present in some DSCT neurones gave in these cells a slight upward convex trajectory shape, preceding the subsequent ramp decay

A firing behaviour similar to that described in Fig 3 B and C (open circles) was not often found in DSCT neurones (see however Fig 9) However in other central neurones such as the rubrospinal neurones (Hultborn Murakami and Tsukahara, to be published) and cells belonging to the ventral spinocerebellar tract (VSCT) such behaviour is more easily found, as illustrated from a VSCT neurone in Fig 5 Note in Fig 5 A the marked upward convex ramp and its rotation with increasing current In the corresponding f/I relation in Fig 5 B the upward concavity is hardly present and is followed at rather low frequencies by an upward convex shape as in the model version in Fig 3 B

The first interval f/I relation in DSCT neurones is then descriptively very similar to that found in motoneurones with a 'primary secondary and 'tertiary range of firing Despite this similarity the underlying mechanisms are not quite the same, as also unveiled by the different trajectory behaviour between motoneurones and DSCT neurones with

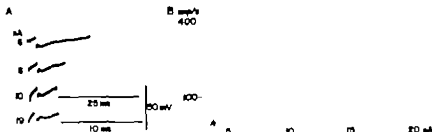


Fig 5 Trajectory shape and first interval f/I relation for a VSCT neurone In the f/I relation in B is given for each current strength the average value (n = 4–6) Compare the upward convex ramp in A with that in Fig 3 B and the corresponding f/I relation in B with that in Fig 3 C (open circles) (Gustafsson, unpublished)

the absence of the scoop inversion (secondary range trajectories) in the latter cells The particular role of the DD in the "DSCT model for the high frequency firing and for the shape of the subsequent trajectories (compare the ramp shape in Fig 3 A and B) should be recognized

B Computed adaptation in the neurone model

In central neurones studied (Kernell 1965 b Eide et al 1969 Koike et al 1970 Baker and Precht 1972) there is a rapid decrease in discharge frequency after the application of a constant current pulse (adaptation) The AHP summation has been considered to be of major importance for this adaptation (Kernell 1969 1972 Calvin and Schwindt 1972) In α-motoneurones and DSCT neurones the adaptation has been shown to be most pronounced at higher currents and to be largely over within the first few interspike intervals (Kernell 1965 b Eide et al. 1969) From the experimental evidence given in the preceding section, the AHP summation in the neurone model was as a first approximation considered to be algebraical To account for the variability from algebraical summation found in real neurones e g the AHP depression a correction factor was also introduced. In this section the results from computations based on this model will be taken up Since the adaptation is largely over within the first interspike intervals, the computations were only performed for the first three intervals following the onset of a current pulse

much the same way as the scoop given by the $G_K(t)$ plateau in the "Mn" model) around the pivot point given by the falling phase of the spike The corresponding f/I relation (Fig 3 C open circles) did, however display the same sigmoid shape as with a DD (filled circles) but in a much more compressed form Also with this model version there was no direct correlation between trajectory shape and f/I shape (compare Fig 3 B and 3 C open circles)

In previous investigations (Kuno and Miyahara 1968) the first interspike interval f/I relation in DSCT neurones was only studied in the higher frequency range, where an upward convex relation, as that demonstrated by the model, was found The trajectory records given displayed the same upward convex-concave-linear shape as given by the DD-scoop-ramp sequence in the computations in Fig 3 A In the present study the first interval f/I relation was examined in 11 DSCT neurones over a much larger frequency range, especially at the lower frequencies and the same sigmoid shape as found in Fig 3 was present *in all these neurones (Paper V) Due to the invariable presence of a* DD both the f/I relations and the trajectories corresponded better to the model version in A than in B The first interval trajectories and the corresponding f/I relation from a typical DSCT neurone is shown in Fig 4 ("tertiary range not illustrated) Observe in Fig 4 A that the trajectory modifications with increasing current are very similar in this neurone to that of the model in Fig 3 As in the model, no correlation between the trajectory behaviour and f/I shape was found In none of these 11 cells was there any sudden jump or discontinuity in the f/I relation or a sudden change in trajectory shape The small $G_K(t)$ plateau probably present in some DSCT neurones gave in these cells a slight upward convex trajectory shape, preceding the subsequent ramp decay

A firing behaviour similar to that described in Fig 3 B and C (open circles) was not often found in DSCT neurones (see however Fig 9) However in other central neurones such as the rubrospinal neurones (Hultborn, Murakami and Tsukahara, to be published) and cells belonging to the ventral spinocerebellar tract (VSCT) such behaviour is more easily found, as illustrated from a VSCT neurone in Fig 5 Note in Fig 5 A the marked upward convex ramp and its rotation with increasing current In the corresponding f/I relation in Fig 5 B the upward concavity is hardly present and is followed at rather low frequencies by an upward convex shape as in the model version in Fig 3 B

The first interval f/I relation in DSCT neurones is then descriptively very *similar to that found in motoneurones* with a 'primary secondary and 'tertiary range of firing Despite this similarity the underlying mechanisms are not quite the same, as also unveiled by the different trajectory behaviour between motoneurones and DSCT neurones with

for the later intervals No crossing between the second and third interval f/i relations was present, demonstrating the role of the $G_K(t)$ plateau for that behaviour (Such a crossing could, however also be present if the first interval had "jumped" to the peak of the DD) In the computed trajectories the DD reached a less depolarized voltage and the scoop was more pronounced for the later intervals

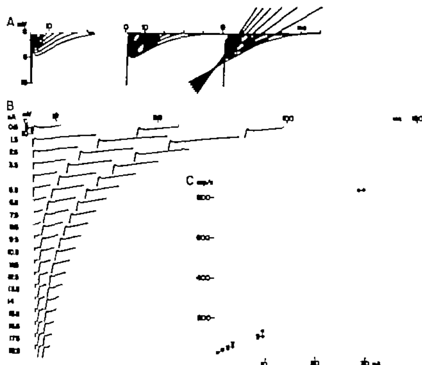


Fig 6 Trajectory shape and *f/i relations* for the first three intervals in the "DSCT" model with algebraical summation A First, second and third interval trajectories are shown superimposed for each interval on a common starting point The straight lines are fitted to the ramp slopes by eye B Same trajectories as in A but displayed sequentially C Relation between the current injected and the reciprocal of the first (triangles) second (open circles) and third (filled circles) intervals For details see text

3 Computation without conductance plateau and with AHP depression Due to the presence of an AHP depression in DSCT neurones the same computation was performed after multiplication of the second and third interval AHP $G_K(t)$ with a factor of 0 5 (Fig 7) The second and third interval f/i relations then derived displayed the same general course as for algebraical summation with linearization and increase in primary -to-'secondary range transition level, but were displaced to the left in the graph crossing the first interval f/i relation twice At low frequencies (< 100 imp/s) the second and third *interval f/i relations were to the left of the first interval f/i* relation, i e there was an initial firing acceleration With higher current the first interval f/i relation was crossing the other two relations i e indicating an initial adaptation However with still higher current, first the second, then the third interval were again crossing the first interval, i e representing again an initial firing acceleration. In the corresponding trajectory records the peak of the DD reached a more depolarized level the scoop was less pronounced and the ramp less steep for the later intervals than for the first interval (see Fig 7 A) i e the contrary from the trajectory behaviour with algebraical summation

Among the 11 DSCT neurones analysed for the three first intervals firing behaviour both patterns described above were found (Fig 8 and 9) Note, however that the second and third interval f/i relations are somewhat more displaced to the right in respect to the first interval f/i relation in the experimental relations than in the model, i.e. the adaptation is somewhat more pronounced or acceleration less pronounced in the real neurones than in the model This phenomenon is most likely caused, as was also described for motoneurones (Paper II) by the fact that the voltage displacement given by a constant current pulse does not remain constant but decays in time due to a still unknown process (Ito and Oshima 1965 Nelson and Lux 1970) giving a difference in threshold current between the first few spikes In three other neurones with marked AHP depression the firing acceleration was only found at the high frequency region and not at the lower frequencies (< 100 imp/s) The f/i slopes in the low frequency region for the second and third intervals were, however in these neurones steeper than for the first interval demonstrating that the adaptation in these neurones was only caused by the current threshold difference described above shifting the f/i curves to the right In the remaining six neurones analysed, no firing acceleration was found at any frequency level and the second and third interval f/i slopes were also less steep than for the first interval Only in one of the cells analysed was there a tendency for a second third interval crossing as that demonstrated for motoneurones A crossing between the first and the second-third interval f/i relations as described above, was not found in the motoneurones analysed.

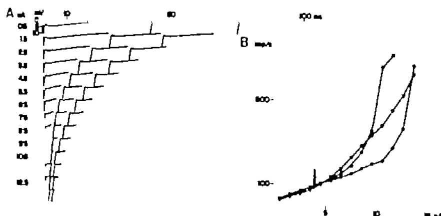


Fig 7 Trajectory shape and f/i relations for the first three intervals in the "DSCT" model with AHP depression A Trajectories for the three intervals displayed sequentially B Relation between current injected and the reciprocal of the first (triangles) second (open circles) and third (filled circles) intervals For details see text

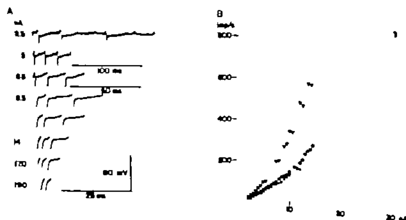


Fig 8 Trajectory shape and f/i relations for the first three intervals in a DSCT neurone In the plot is given the average value of 4–6 trials at each current strength (500 ms pulses at a frequency of 0 2/s) Same symbols as in Fig 7 and 5 For details see text

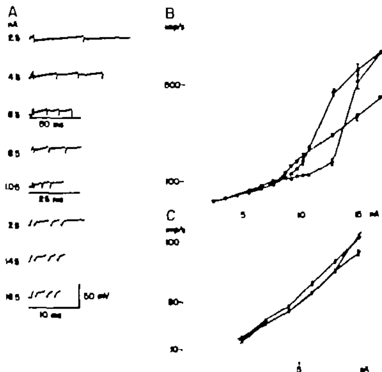


Fig 9 Trajectory shape and f/i relations for the first three intervals in a DSCT neurone In the plot is given the average value ± S E of 4–6 trials at each current strength (500 ms pulses at a frequency of 0 2/s Same symbols as in Fig 8 For details see text In C is shown an enlargement of the lower part of the graph in B

Thus the predictions of the model do not only account for the general features of the initial adaptation in DSCT neurones such as the increase in linearization of primary range and the transition level to secondary range with successive intervals,but also more specifically for the presence of the two phases of firing acceleration given by the AHP depression The difference in the firing behaviour between motoneurones and DSCT neurones already found for the first interval is also more accentuated (lack of second-third interval crossing in DSCT neurones and lack of first-second interval crossing in motoneurones) and is apparently accounted for by the difference in AHP properties

C Computed steady state firing

As demonstrated in Paper III a model based on an exponential $G_K(t)$ decay and an algebraical summation will for the steady state firing, i. e when the adaptation is completed display a slightly upward convex, almost linear relation up to very high frequencies Such a model will thus not display a secondary' range On the other hand, in the presence of a pronounced $G_K(t)$ plateau the steady state f/i relation will bend upwards at higher frequencies thus displaying a secondary range of firing A model based on the AHP $G_K(t)$ time course and algebraical summation is then able to explain the original difference described between motoneuronal firing behaviour and that of the DSCT neurones namely the presence of a secondary range in some of the former cells and its absence in the latter cells (Kernell 1965 c Eide et al 1969)

In the steady state firing in motoneurones with a pronounced $G_K(t)$ plateau the secondary range should be reached when the frequency exceeds that corresponding to the end of the plateau phase (or approximately the 1/time–to–peak AHP) It is however reported that the transition frequency also for the steady state firing can be appreciably lower than this limiting frequency (Kernell 1965 d) Moreover the ratio between the steady state secondary /'primary f/i slopes can be appreciably higher in real neurones (Kernell 1965 c) than that given by the model, thus implicating an additional mechanism for the secondary range of firing in motoneurones It should, however be recalled that the algebraical summation is only a first approximation to the AHP summation mechanism in motoneurones and, in fact, in many of the motoneurones analysed the summation was appreciably less than algebraical at shorter intervals In fact, if the summation is less than algebraical at shorter intervals and algebraical at longer intervals, i e if the correction factor is not constant but decreases with decreasing interval, then the secondary range would be expected to start at lower frequencies and deviate more from the 'primary' range f/i slope than that predicted by algebraical summation Motoneurones and DSCT neurones may then not only differ with respect to the $G_K(t)$ time course but to some extent also to the AHP summation. However in the present investigations the f/i slope of two DSCT neurones was also somewhat steeper at higher frequencies than at lower In motoneurones with less developed $G_K(t)$ plateau the firing would be more similar to that of the DSCT neurones i e the steady state secondary' range could even be (if algebraical summation) hardly present in such cells This prediction then fits well with the recent observation (Schwindt 1973) that motoneurones lacking steady state secondary range even up to very high discharge frequencies have a shorter AHP duration and thus likely a shorter time–to–peak AHP (Kernell 1965 d) and display more adaptation than neurones giving steady state secondary range

Even if the firing behaviour of a model based on the AHP conductance time courses (and the DD current) is fairly similar to that found in real neurones there are several aspects of the firing in real neurones not accounted for by such a model

The adaptation in the model is given by the AHP summation and is completed within the first few interspike intervals The presence of adaptation in cells displaying an AHP depression and the appreciably longer adaptation found in both motoneurones (Granit et al 1963 b Kernell 1965 b) and DSCT neurones (Eide et al 1969) demonstrates that other mechanisms must be included. As discussed previously the "sag" in the voltage given by a constant current pulse is likely to give rise to the current threshold difference between the first few spikes and thus to the adaptation found in cells with AHP depression. A longlasting adaptation has also been found in invertebrate neurones (Sokolove and Cooke 1970) and has there been attributed to the activation of an electrogenic sodium pump The existence of such a sodium pump has also been verified in vertebrate neurones by the injection of Na^+ ions through the recording microelectrode (Ito and Oshima 1965 a, b Koike Mano, Okada and Oshima 1972) In pyramidal tract (PT) neurones a slower hyperpolarizing component of the AHP appearing after a long (> 20 spikes) train of spikes at high frequency has been attributed to the activation of this sodium pump and has been considered as a mechanism for a longlasting adaptation

In the present investigations the afterpotentials following long spike trains of high frequency were studied in 12 DSCT neurones and a typical finding is exemplified in Fig 10 from two neurones The posttetanic hyperpolarization (PTH) so evoked, was hardly noticable with spike trains below a few seconds (100 Hz frequency) and increased thereafter in proportion to the train duration The peak voltage of the PTH was usually not reached until a couple of seconds after the end of the train and decayed thereafter slowly during 50–100 s There was no apparent conductance increase during the PTH, as judged from the voltage drop caused by current pulses (see Fig 10 B) indicating that it is caused by an active process such as a sodium electrogenic pump This PTH needs then more spikes and lasts considerably longer than that found in PT neurones Its time course is however fully compatible with that expected from an electrogenic pump (Ito and Oshima 1965 a b Koike et al. 1972) The slow onset and the number of spikes needed to evoke this PTH would indicate that it hardly can account for much of the adaptation in motoneurones and DSCT neurones during the first few seconds of a discharge, but rather could be responsible for the later adaptation in

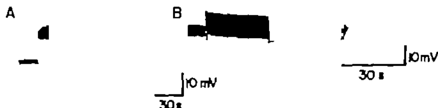


Fig 10 Post-tetanic hyperpolarization (PTH) in DSCT neurones In A and B are shown the hyperpolarization following a longlasting tetanic (100/s) antidromic (cerebellum) activation in two DSCT neurones Observe in A the increase in PTH with increased number of spikes In B can be observed the constancy in the voltage deflection given by a train of constant current pulses throughout the PTH For further details see text.

motoneurones described by Granit et al (1963 b) and also found in DSCT neurones (Gustafsson Lindström Takata and Zangger to be published)

In the model there is no frequency limiting process implying that the steady state frequency can reach the highest values While the steady state firing in DSCT neurones can reach very high frequencies (up to 600 imp/s; Eide et al 1969) the steady state firing in motoneurones in response to constant current injection never exceeds 150–200 imp/s (Kernell 1965 c) and very often the steady firing fails at considerably lower frequencies Whether this difference is caused by the larger amount of current needed to reach high frequencies in motoneurones than in DSCT neurones by a higher sensitivity in motoneurones for inactivation given by insufficient repolarization between the spikes or by the presence of the brief overswing in DSCT neurones preventing inactivation, is at present not known It should be noted that the DSCT neurones invariably despite the apparent injury of these cells upon penetration (Eide et al 1969) responds to constant current injection with a continuous discharge (see also Eide et al. 1969) and do not show phasic behaviour as some motoneurones (Mishelevich 1969) indicating some fundamental difference between DSCT neurones and at least some motoneurones To which extent the inactivation contributes to the adaptation in continuously firing neurones is unclear

In the model only the K conductance is allowed to summate or change with repetitive activation. In motoneurones (Kernell 1964) and in DSCT neurones (Paper IV) the DD can also increase with successive spikes a feature which in one DSCT neurone investigated was of decisive importance for its firing behaviour In this neurone the AHP following a single spike was hardly noticable, increasing slowly with successive spikes This allowed the DD to overcome the AHP for the first few spikes and the neurone was subsequently responding with a short burst of spikes terminated by the AHP summation, the bursts increasing in frequency with increasing current The DD increase served thus as an intrinsic mechanism for burst firing emerging in the presence of a minute AHP This multiple-spike response of the DSCT neurones was somewhat similar to that found in the pyramidal cells in hippocampus (Kandel and Spencer 1961) or in neurones in the inferior olive nucleus (Crill 1970; see also Calvin 1974) In the pyramidal cells the burst was however terminated by a sodium conductance inactivation and not by the AHP summation

A AHP and firing control

It is generally agreed that for the understanding of the properties of the rhythmic firing in neurones with their low frequency firing ability and their large frequency range, long time constant processes not included in the Hodgkin-Huxley equations for the squid axon (Hodgkin and Huxley 1952) must be visualized. Since little is known about such long time constant processes models trying to simulate the firing behaviour of central neurones have either been formal, including a long decay of a simple threshold function (Kernell 1968 Pertile and Harth 1971) or been modifications of voltage clamp equations from squid axon (Shapiro and Lenherr 1972) or myelinated nerve fibre (Kernell and Sjöholm 1972, 1973) However as stated in the Introduction, even such an elaborated model as that of Kernell (1972, 1973) is still not accounting for the specific firing behaviour of central neurones It may be asked if this difference is caused by the specific properties of the processes underlying the afterpotentials in neurones not accounted for by modified voltage clamp equations from axons or by the influence of other factors such as the spatial distribution, not easily foreseen from the axonal properties

In the Introduction two alternative hypotheses for explaining the firing behaviour of central neurones were brought up On one hand, the firing behaviour was determined by the time course of the refractoriness, on the other the changes in spatial distribution of the AHP was considered to be critical. The results presented now are clearly favouring the former view The trajectory modifications and f/I relations given by the model based on fixed postspike conductance processes are very similar to those found in the real neurones Thus neither the trajectory behaviour described by Schwindt and Calvin (1972) and Schwindt (1973) nor the sudden change in trajectory shape when entering the 'secondary' range, described by Kernell (1965 c) are, in fact, indicating that any change in spatial distribution of the AHP given by increasing current injection should occur The difference between the f/I relations and trajectory behaviour in motoneurones and DSCT neurones seems also to be a consequence of a difference in the respective AHP conductance properties The effect of synaptic currents during rhythmic firing has also recently been re-examined (Schwindt and Calvin 1973 a) and no effects such as those reported by Granit et al (1966 b) could be found It seems therefore that very little evidence exists that changes in the spatial distribution of the AHP should play any decisive role in firing regulation

This conclusion does however not mean that the spatial distribution of the antidromic invasion could not be of importance for the firing behaviour An alternative view on the *effect of a spatial factor* was recently forwarded in a preliminary note (Mauritz, Schlue, Richter and Nacimiento 1973) In this paper it was suggested that the plateau in the conductance time course could be explained by a limited dendritic invasion during this phase, thus indirectly re-emphasising the role of the neuronal geometry For example, the lack of 'secondary range in DSCT neurones could then be a consequence of a higher safety factor for impulse transmission in these cells as suggested by Eide *et al* (1969) The hypothesis that the plateau is given by a limited dendritic invasion is neither supported nor clearly invalidated by the present findings It was, however stated in that note that "the limited dendritic invasion increases with each additional spike until a maximum is reached for the corresponding current strength" This postulated increase in dendritic invasion with the later intervals removing the plateau, seems somewhat in conflict with the successive changes in the first, second and third interval trajectories during adaptation displayed by real neurones and well fitting with *the algebraical summation* *Moreover* the lack of effects of synaptic currents on the f/i relations reported by Schwindt and Calvin (1973 a) and the relative stability of the plateau phase with subthreshold polarizations and with suprathreshold currents seems also to indicate that the plateau is more fixed than expected if governed by a spatial factor (see also below)

A model for repetitive firing regulation in motoneurones based on fixed only time-dependent postspike conductance changes has recently been criticized from another angle than from the consideration of spatial factors (Schwindt and Calvin 1973 b) In that paper it was suggested that the firing in motoneurones is regulated by a time and voltage dependent potassium conductance and a possible subthreshold sodium conductance change which is time and voltage dependent This latter conductance should be responsible for the development of the secondary range trajectory shape and subsequently to the secondary' range of firing

From scrutiny of the experimental results reported by Schwindt and Calvin (1973 b), it is clear that only those results concerning the conductance measurements are opposed to that which would be predicted by the model In fact, due to the presence of the AHP summation no *linear relation between* scoop depth and current injected would be found in the model, a behaviour thus confirmed in real neurones by Schwindt and Calvin (1973 b) Also the findings presented in *the downstep experiments* are clearly in line with the model behaviour

The model would, however not predict the linear *relation between the scoop depth* and *the inverse of* current injected reported This is no fault of the model

since such a linear relation should only be present in the motoneurone in Fig 4 B (Schwindt and Calvin 1973 b) if the threshold current (which should be of no interest in this respect) is included

On the other hand, the model would not predict the findings that the interspike interval (ISI) conductance should be lower (or the same) during a short ISI than during a long ISI or at resting level, and that it should be lower than the resting conductance in the later part of the ISI According to Schwindt and Calvin these results should indicate the presence during the ISI of a voltage dependent sodium conductance evoked by the spike (see also Discussion, Paper I)

There are, however some evidence that the con ductance time courses given by Schwindt and Calvin should not be taken for granted. In fact, other authors (Mauritz et al, 1975) have recently reported measurements of conductance time courses during rhythmic firing and found similar time courses as that during resting conditions i e with conductance increase and presence of a conductance plateau. Moreover in the experiments by Schwindt and Calvin it was not always possible to maintain the bridge balance, which could easily give erroneous results since an incompensation should not only affect the absolute conductance but also its time course It should also be noted that the findings of constant conductance in the early part of the ISI with increasing currents seems to be in contradiction to the above-mentioned finding of a nonlinear relation between scoop depth and current injected. There are, however no reasons to doubt that the huge increases in pulse height occurring at the very end of the ISI to some extent can be real and be produced by the activation of a sodium conductance giving the succeeding spike Such a sodium activation is in fact, also indicated by the upward deviation of the experimental trajectories at the very end of the ramp (see, for example, Fig 2 Schwindt and Calvin 1972) not present in the computed trajectories The im portance of that conductance for firing regulation would however be far from that suggested for the hypothetical sodium conductance of Schwindt and Calvin

In the present proposed model the K conductance underlying the AHP is treated as completely separated from the K conductance underlying the falling phase of the spike This separation was suggested for motoneurones already by Eccles (1957) and is also a feature of the Kernell model (Kernell and Sjöholm 1972) The AHP K conductance was considered by Eccles to increase slowly after the spike, reaching a late peak, corresponding to the peak of the AHP voltage, thereafter *slowly decaying In the Kernell model the AHP K conductance displays* a simple exponential decay starting during the descending phase of the spike From the finding of a selective depression of the AHP evidence is now at hand that the AHP K conductance in fact is separated from the K conductance underlying the falling phase of the spike From the conductance computation and measurements in motoneurones it would be tempting to accept the *suggestion of Eccles However from the* time courses of the depressed AHPs in DSCT neurones it seems, however as if the whole computed time course, including also the initial fast $G_K(t)$ decay is belonging to the AHP K conductance

As mentioned above, it was recently suggested that a limited dendritic invasion could account for the plateau, thus giving an explanation for the odd and (especially in motoneurones) perhaps even unlikely (in respect to Hodgkin-Huxley equations) AHP $G_K(t)$ configuration The coupling existing between the time-to-peak AHP (or plateau duration) and the duration of the AHP (or decay time constant) even if somewhat variable (Kernell 1965 d) seems however to suggest that the plateau is given by the time course of the ionic kinetics rather than by a spatial mechanism (see also above) The existence of K conductance systems not included in the Hodgkin-Huxley equations has also been recently described in molluscan neurones (Conner and Stevens 1971 Gola 1974) indicating that other ionic mechanisms than those described for axons can operate in the soma-dendritic membrane of central neurones

The concept of algebraical summation i e that the AHP conductance is not affected by the following spikes and that each spike is evoking the same K conductance was introduced as a first approximation for description of the AHP summation Even if it is clear from the experimental analysis that each spike is not evoking the same K conductance the idea that the AHP conductance is not affected by the succeeding spikes has been successful for explaining the successive trajectory changes during adaptation in motoneurones and of the summation-depression pattern and initial high frequency firing acceleration in DSCT neurones In the Kernell model an AHP summation can be present if the rate constant for the AHP K conductance is chosen so that the peak value of that conductance is not reached during the first spike Due to the con-

ditioning effect of the remaining conductance, a higher one will be evoked by the next spike This model will thus explain neither the long-lasting depression nor the difference in $G_K(t)$ time course between the AHPs following one and two spikes

In the model there will be an equivalence between current injected through a microelectrode and currents given by synaptic conductance changes Even if such an equivalence exists in many motoneurones under synaptic drive from different sources (Schwindt and Calvin 1973 a, Granit et al 1966 a, b) a change in the f/i slope in both the primary' and the secondary range of firing has been reported (Kernell 1965 a, Granit, Kernell and Lamarre 1966 b Shapovalov Kurchavyi and Strogonova 1966 Shapovalov 1972) If this behaviour is caused by the interaction of synaptic and AHP conductance changes in the dendritic tree (Kernell 1971) or by other factors (Granit et al 1966 b) is at present unknown.

C Descriptive terminology

Many previous studies of the rhythmic firing in motoneurones have mainly been concerned with describing the f/i relations and trajectory behaviour The f/i relations and trajectories have been linearized and related to each other via simple geometrical schemes (Schwindt 1973) Even if it was appreciated that these descriptions mainly served as con straints for future modelling conclusions regarding possible underlying mechanisms based on these simplistic schemes were drawn (Schwindt and Calvin 1972 Schwindt 1973) The different trajectory behaviour found between motoneurones displaying e g."stereotyped" ramps and some other CNS neurones displaying e g ramp slope changes were then taken as indications of different firing mechanisms (Schwindt and Calvin 1972, Schwindt 1973)

The present studies have instead started the analysis from a different angle, deriving the f/i relations and trajectories from a postulated underlying mechanism, covering not only motoneurones but also with minor modifications DSCT neurones The firing then derived displayed characteristics well within the general framework given by previous investigations There were, however many deviations in the model behaviour from the simple schemes earlier suggested The f/i relations were not linear and thus not easily dividable in different firing ranges and the trajectory changes were not as clear cut as described. No easy correlation could be made between various segments of the f/i relations and of the trajectories especially not when comparing the various model versions It is apparent from the presented results that the same more complex firing behaviour in fact is present also in the real neurones

and that thus the same difficulty in imposing simple firing range subdivision and trajectory description is present also for them. Most of the nomenclature earlier given is thus not helpful when interpreting the underlying mechanism but is rather obscuring it. In fact, different f/i shapes can be given by the same underlying mechanism and different underlying mechanisms can give the same f/i shape Different trajectory shapes can be given by the same underlying mechanism and the same trajectory shape can be given by different underlying mechanisms These statements are exemplified by considering that the f/i relation and the ramp behaviour given by an exponential decay is much dependent upon the initial phase of the AHP and on the DD The upward bending of the f/i relation or a 'secondary range can result from an exponential decay and a plateau. The upward convex trajectory shape can originate from a DD a plateau and even an exponential $G_K(t)$ decay

Even so it is obvious that a correlation between the underlying mechanisms the f/i shape and the trajectory shape exists and thus that a scrutiny of e g the trajectories will disclose both the expected f/i relations and the underlying mechanism The present results do however demonstrate that in such a correlation the whole sequence of trajectories must be considered as a unity and the interpretation of a trajectory must be related not only to its position in the interspike interval and when it appears with increasing current but also in relation to the trajectory shapes preceding it By such an analysis the f/i relations could be predicted, as well as the underlying mechanism Such predictions given by the model analysis and verified in real neurones are easily exemplified For example, the presence of a small DD an early deep scoop and an upward convex ramp is indicating from the model an almost linear to upward convex f/i relation already for the first interval, a finding also verified in real neurones An expressed DD and a shallow early scoop followed by an almost linear ramp (changing in slope with increasing current) gives the sigmoid first interval f/i relation and a slightly upward convex almost linear steady state f/i relation The presence of an upward concave scoop gradually shifting to upward convexity is indicative of a conductance plateau, possibly giving firing jumps for the first interspike intervals and an upward deviation of the f/i relation at frequencies exceeding 1/time-to-peak AHP

D Other CNS neurones

In a few other CNS neurones some data are available on the AHP and the firing properties These neurones can, interestingly enough, be subdivided into two groups grossly comparable to α-motoneurones and DSCT neurones respectively On one hand, the trochlear (TMn;

Baker and Precht 1972) and Facial (FMn) motoneurones (Kitai et al 1973) and the pyramidal tract (PT) cells (Takahashi 1965) display AHPs with rather long time-to-peak (6-10 ms in TMn and FMn and appreciably longer in PT cells) showing summation and no depression with repetitive activation. A steady state secondary range is also present in PT neurones (Koike et al. 1970) and an upward concave f/i relation can also be found in TMn (Baker and Precht 1972; FMn not investigated in this respect) No increase in firing rate with time has been found in TMn and slow PT cells In fast PT cells, however a firing acceleration comparable to that in DSCT neurones at a short first interspike interval has been found (Koike et al 1970) On the other hand the rubro- (Tsukahara, Toyama and Kosaka 1967) and vestibulospinal (Ito Hongo Yoshida, Okada and Obata 1964) neurones exhibit an AHP with a very short time-to-peak (1-3 ms) i e even shorter than DSCT showing no summation (Tsukahara et al 1967 Ito et al. 1964) or in the rubrospinal neurones even an AHP depression (Hultborn, Murakami and Tsukahara, to be published) i.e a behaviour very similar to that in DSCT neurones In the rubrospinal neurones the first interspike interval firing is very similar to that shown in Fig 3 and in the steady state secondary range is usually not found (Hultborn, Murakami and Tsukahara, to be published)

E Functional implications

In the preceding section it was suggested that neurones discharging repetitively in a steady fashion could be subdivided into at least two groups with respect to the AHP properties and the firing behaviour A coupling between the firing behaviour displayed by neurones belonging to these two groups and their function has been made by previous authors (Kernell 1965 d, 1966 Eide et al 1969) For the α-motoneurones it was suggested (Kernell 1965 d) that the presence of a secondary range in the steady state firing could be present as a compensation for the decline in the muscle tension-firing frequency relation at higher frequencies This coupling between firing behaviour and muscle properties is then well in line with the fact that the other neurones grouped together with the α-motoneurones except the PT cells are directly connected to muscles It has however been questioned to which extent the motoneurones in steady firing are reaching these high frequencies and thus the usefulness of this mechanism (Schwindt 1973 see, however Kernell 1966) On the other hand, high discharge frequencies are more easily evoked initially in the discharge, a behaviour which was then considered (Kernell 1966) in relation to the fact that a muscle requires much higher stimulus rates for a maximal rate of rise of tension than for the maintainance of a maximal tetanic contraction (Buller and Lewis

1965) That the initial firing behaviour could be of importance in relation to the muscle properties is more emphasized by the catch property (Wilson and Larimner 1968) also found in mammalian muscles (Burke, Rudomin and Zajac III 1970) i e that the tension given by a low frequency firing is greatly enhanced during a considerable time by an initial short interspike interval. The AHP mechanism in motoneurones with a $G_K(t)$ plateau, giving rapid shortening of the first interspike interval, and an AHP summation giving a low succeeding frequency is then well suited for use of this muscle property The steady state secondary range might then rather in that context, be considered as a spin off' behaviour *An initial short interspike interval followed by* a low frequency discharge is in fact, also the manner in which fast movements are initiated in human muscles (Gurfinkel, Mirskii Tarko and Surguladze 1972) As in the motoneurone model and as in the real neurones this short first interval is characteristically also followed by a longer second than third interval By comparing the records in Fig 1 (Gurfinkel et al 1972) where the first, second and third intervals are 72 12 and 18 imp/s respectively and e g the graph in Fig 7 B Paper III, where the corresponding intervals at 20 nA are 64 13 5 and 15 5 imp/s it is clear that the AHP is quantitatively capable of producing the firing behaviour described by Gurfinkel et al. without any contribution from other mechanisms

The linear steady state f/i relation found in DSCT neurones is easily imagined as appropriate for their function, since with such a relation a given synaptic input will give the same frequency increase regardless of other synaptic activity (Eide et al 1969) Since these neurones are most likely firing more or less continuously i e being steadily adapted, the upward deviations or secondary ranges found for the first interspike intervals with constant current injections might then rather be a spin off' behaviour just indicating the ingenious manner in which a linear steady state f/i relation is achieved through a linear summation of exponential decays A similar reasoning might also be applicable to the rubro- and vestibulospinal neurones also not directly coupled to the muscles The significance of the AHP depression is not easily understood It is however clear that the depression is a very effective means of converting the f/i shape from the sigmoid shape of the first *interval to the "desired steady state linearity* without much adaptation, which might be an unnecessary and even unwanted feature of neurones not coupled to muscles

It was recently suggested that the trajectory behaviour in motoneurones during primary range firing was well suited for maintaining a constant firing behaviour in the presence of accomodation and synaptic activity (Schwindt 1973) This suggestion was based on the implicit assumptions that the ramp in motoneurones was stereotyped

and that the input resistance was constant throughout the ramp Since neither the ramp is stereotyped nor the resistance constant this suggestion seems hardly warranted.

In the present studies the AHP properties in both α-motoneurones and the *dorsal spinocerebellar tract* (DSCT) *neurones were investigated* in the cat A neurone model based on the AHP mechanisms and on some simplifying assumptions of the neurone properties was then derived. The firing behaviour of this model was computed and a comparison between the model firing and the firing behaviour of the real neurones was performed

From these investigations the following conclusions were drawn

1 The AHP in DSCT neurones is given by a K conductance process as earlier described for motoneurones (Coombs et al 1955) and not by a sodium electrogenic pump (Kuno et al 1970) or by a sodium conductance decrease (Eide et al 1969)

2 The K conductance underlying the AHP is separated from that giving rise to the falling phase of the spike as suggested previously (Eccles 1957) In motoneurones this K conductance describes a complex time course with an initial fast decay a plateau phase and a subsequent slow exponential decay In DSCT neurones the peak AHP voltage is substantially smaller and is reached earlier than in motoneurones This is correlated with a lack or poor development of the plateau phase in the conductance time course in DSCT neurones The AHP conductance was found to be only little affected by subthreshold potential variations Equations describing the conductance time course in both motoneurones and DSCT neurones were given

3 In both motoneurones and DSCT neurones the AHP summation can as a first approximation, be described as an algebraical process The absolute summation can, however be larger or smaller than the algebraical summation. Some DSCT neurones besides showing AHP summation, can also present a temporal depression of AHP i e the AHP following two spikes is less than that following a single spike

4 The frequency-current (f/I) relation in the model based on the *AHP conductance time course in motoneurones displays* the three firing ranges found in real motoneurones (Kernell 1965 c Schwindt 1973) With increasing current injection the interspike voltage trajectories are also undergoing the same peculiar modifications as described for real motoneurones (Schwindt and Calvin 1972 Schwindt 1973) A re-examination of the first interspike interval firing in real motoneurones revealed features predicted by the model, not observed before It is concluded that the model is well simulating the firing behaviour of the first interspike interval in motoneurones

5 Implemented with an algebraical summation of the AHP conductance following successive spikes the same model simulates well the successive changes in the f/i relations and the interspike voltage trajectories found in real motoneurones for the initial intervals after the onset of a constant current The steady state f/i relation of the model can also display an upward deviation from linearity at higher current intensities i e a secondary' range of firing as described for real motoneurones (Kernell 1965 c) It is concluded that the AHP conductance is the decisive factor for determining the f/i relation as well as the interspike voltage trajectories in motoneurones

6 The firing behaviour of a neurone model based on the AHP conductance time course in DSCT neurones and on a shortlasting depolarizing current giving rise to the delayed depolarization simulates adequately the first interspike interval f/i relation and trajectory behaviour in these cells as experimentally determined in the present investigations Even if the f/i relation in DSCT neurones (and the model) could be descriptively subdivided into three firing ranges as in motoneurones the underlying mechanism for this behaviour is not quite the same as for motoneurones as also unveiled by the somewhat different trajectory behaviour

7 The firing behaviour of the same neurone model was analysed after implementing the features of AHP summation and depression. Firing behaviour thus predicted was also found in real DSCT neurones, most notably the two phases of firing acceleration given by the AHP depression. The steady state f/i relation of the model was linear, as demonstrated earlier for DSCT neurones (Eide et al 1969) It was concluded that also in DSCT neurones the AHP is of decisive importance for the firing regulation.

8 The model based on the AHP conductance thus not only has met with the constraints imposed from previous studies on repetitive firing in motoneurones (Kernell 1965 c Schwindt and Calvin 1972, Schwindt 1973) but also predicted firing behaviour subsequently confirmed in real neurones However it was recognized that the model is insufficient in accounting for several aspects of the firing behaviour in real neurones Additional mechanisms such as membrane non linearities sodium conductance inactivation, sodium electrogenic pumps and the neuronal geometry are discussed.

9 The descriptive terminology for the trajectory behaviour (Schwindt and Calvin 1972, Schwindt 1973) and for f/i relations (Kernell 1965 c Schwindt 1973) are discussed in relation to the model behaviour It is concluded that this terminology in some respects is inconsistent and can give rise to misinterpretations of underlying mechanisms.

10 The results in the present investigation are discussed in relation to findings in other central neurones A distinction in AHP properties and firing behaviour between neurones with and without connexion to muscles is recognized and the difference is discussed

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to Professor Anders Lundberg for introducing me into this field of neurophysiology and for his patience and continuous support during the performance of this work.

I also wish to thank Fausto Baldissera, Sivert Lindström Mitsuru Takata and Peter Zangger for very stimulating collaboration

Thanks are also due to Erling Eide with collaborators and Yngve Källström for invaluable technical constructions to Rauni Larsson for excellent assistance in the experiments and during the preparation of the manuscripts to Berit Nilsson for expert secreterial work and to the photographer Tomas Palm

The valuable help of Serge Rossignol for scrutinizing the English text is gratefully acknowledged.

This work was supported by the Swedish Medical Research Council (project No 94) the Medical Faculty University of Göteborg and Svenska Sällskapet för Medicinsk Forskning

ADRIAN E D and Y ZOTTERMAN The impulses produced by sensory nerve-endings Part 2 The response of a single end-organ J Physiol (Lond) 1926 61 151-171

BAKER R and W PRECHT Electrophysiological properties of trochlear motoneurons as revealed by IVth nerve stimulation Exp Brain Res 1972 14 127-157

BROCK L G J S COOMBS and J C ECCLES The recording of potentials from motoneurones with an intracellular electrode J Physiol. (Lond.) 1952 117 431-460

BURKE, R E P RUDOMIN and F E ZAJAC III, Catch property in single mammalian motor units Science 1970 168 122-1.4

CALVIN W H Three modes of repetitive firing and the role of threshold time course between spikes Brain Res 1974 69 341-346

CALVIN W H and P C SCHWINDT Steps in production of motoneuron spikes during rhythmic firing J Neurophysiol. 1972 35 297-310

CONNOR, J A and C F STEVENS Prediction of repetitive firing behaviour from voltage clamp data on an isolated neurone soma J Physiol (Lond.) 1971 213 31-53

COOMBS J S J C ECCLES and P FATT The electrical properties of the motoneurone membrane J Physiol (Lond) 1955 130 291-325

CRILL, W E Unitary multiple-spiked responses in cat inferior olive nucleus J Neurophysiol. 1970 33 199-209

ECCLES, J C The Physiology of Nerve Cells Johns Hopkins Press Baltimore 1957

ECCLES J C R M. ECCLES and A LUNDBERG The action potentials of the alpha motoneurones supplying fast and slow muscles J Physiol (Lond) 1958 142 275-291

EIDE E L FEDINA J JANSEN A LUNDBERG and L VYKLICKY Properties of Clarke s column neurones Acta physiol scand. 1969 77 125-144

GOLA M. Evolution de la forme des potentiels d action par stimulations répétitives Pflügers Arch. ges Physiol. 1974 346 121-140

GRANIT R D KERNELL and R S SMITH Delayed depolarization and the repetitive response to intracellular stimulation of mammalian motoneurones J Physiol (Lond.) 1963 168 890-910

GRANIT R D KERNELL and G K SHORTESS Quantitative aspects of repetitive firing of mammalian motoneurones, caused by injected currents J Physiol (Lond) 1963 a 168 911-931

GRANIT R D KERNELL and G K SHORTESS, The behaviour of mammalian motoneurones during long-lasting orthodromic anti-dromic and trans membrane stimulation J Physiol (Lond.) 1963 b 169 743-754

GRANIT R D KERNELL and Y LAMARRE, Algebraical summation in synaptic activation of motoneurones firing within the 'primary range' to injected currents J Physiol. (Lond.) 1966 a. 187 379-399

GRANIT R. D KERNELL and Y LAMARRE Synaptic stimulation superimposed on motoneurones firing in the secondary range to injected current. J Physiol. (Lond.) 1966 b 187 401-415

GURFINKEL, V S M L MIRSKII A M TARKO and T D SURGULADZE Function of human motor units on initiation of muscle tension. Biofizika 1972 17 No 2 303-310

HODGKIN A L and A F HUXLEY A quantitative description of membrane current and its application to conduction and excitation in nerve J Physiol. (Lond.) 1952 117 500-544

ITO M T HONGO M YOSHIDA, Y OKADA and K OBATA Antidromic and trans-synaptic activation of Deiters neurones induced from the spinal cord Jap J Physiol 1964 14 638-658

ITO M and T OSHIMA Temporal summation of after-hyperpolarization following a motoneurone spike Nature 1962 195 910-911

ITO M. and T OSHIMA, The electrogenic action of cations on cat spinal motoneurons Proc roy Soc B 1964 a 161 92-108

ITO M. and T OSHIMA The extrusion of sodium from cat spinal motoneurons Proc roy Soc B 1964 b 161 109 131

ITO M and T OSHIMA Electrical behaviour of the motoneurone membrane during intracellularly applied current steps J Physiol (Lond.) 1965 180 607-635

KANDEL E R. and W A SPENCER, Electrophysiology of hippocampal neurons II Afterpotentials and repetitive firing J Neurophysiol. 1961 24 243 259

KERNELL, D The delayed depolarization in cat and rat motoneurones Progr Brain Res 1964 12 42-55

KERNELL, D Synaptic influence on the repetitive activity elicited in cat lumbosacral motoneurones by long-lasting injected currents Acta physiol. scand. 1965 a. 63 409-410

KERNELL, D The adaptation and the relation between discharge frequency and current strength of cat lumbosacral motoneurones stimulated by long lasting injected currents Acta physiol. scand. 1965 b 65 65 73

KERNELL D High-frequency repetitive firing of cat lumbosacral motoneurones stimulated by long lasting injected currents Acta physiol. scand. 1965 c 65 74-86

KERNELL, D The limits of firing frequency in cat lumbosacral motoneurones possessing different time course of afterhyperpolarization Acta physiol. scand 1965 d 65 87-100

KERNELL D The repetitive discharge of motoneurones In Muscular Afferents and Motor Control Nobel Symposium I Ed. R Granit. Almqvist & Wiksell, Stockholm 1966 351-362

KERNELL D Repetitive impulse discharge of a simple neurone model compared to that of spinal motoneurones Brain Res 1968 11 685-687

KERNELL, D Effects of synapses of dendrites and soma on the repetitive impulse firing of a compartmental neuron model. Brain Res 1971 35 551-555

KERNELL, D and H SJÖHOLM, Motoneurone models based on 'voltage clamp equations for peripheral nerve Acta physiol scand 1972 86 546-562

KERNELL, D and H SJÖHOLM Repetitive impulse firing comparisons between neurone models based on 'voltage clamp equations' and spinal motoneurones Acta physiol scand 1973 87 40-56

KITAI, S T T TANAKA N TSUKAHARA and H YU The facial nucleus of cat; Antidromic and synaptic activation and peripheral nerve representation Exp Brain Res 1972 16 161-183

KOIKE H N MANO Y OKADA and T OSHIMA Repetitive impulses generated in fast and slow pyramidal tract cells by intracellularly applied current steps Exp Brain Res 1970 11 263 281

KOIKE H N MANO Y OKADA and T OSHIMA Activities of the sodium pump in cat pyramidal tract cells investigated with intracellular injection of sodium ions Exp Brain Res 1972 14 449-462

KUNO M Excitability following antidromic activation in spinal motoneurones supplying red muscles J Physiol. (Lond.) 1959 149 374-393

KUNO M and J T MIYAHARA Factors responsible for multiple discharge of neurons in Clarke s column. J Neurophysiol. 1968 31 624-638

KUNO M. J T MIYAHARA and J N WEAKLY Post tetanic hyperpolarization produced by an electrogenic pump in dorsal spinocerebellar tract neurones of the cat. J Physiol. (Lond) 1970 210 839-855

MAURITZ K -H W R SCHLUE D W RICHTER and A C NACIMIENTO Conductance changes during the afterhyperpolarization and repetitive firing in cat spinal motoneurones Pflügers Arch ges Physiol. 1973 339 Suppl. R73

MISHELEVICH, D I Repetitive firing to current in cat motoneurons as a function of muscle unit twitch type. Exp Neurol. 1969 25 401-409

NELSON P G and R E BURKE Delayed depolarization in cat spinal motoneurons Exp Neurol 1967 17 16-26

NELSON P G and H.D LUX, Some electrical measurements of motoneuron parameters Biophysical Journal 1970 10 55-73

PERTILE G and E HARTH, A model of adaptation based on relaxation phenomena in the neural membrane. Kybernetik 1971 9 189-196

PIERAU F -K M.R KLEE and D S FABER, Temperature effects on spike parameters and resting potential in motoneurons XXV International Congress 1971 9 1344

SCHWINDT P C Membrane-potential trajectories underlying motoneuron rhythmic firing at high rates J Neurophysiol. 1973 36 434-449

SCHWINDT P C and W H CALVIN Membrane-potential trajectories between spikes underlying motoneuron firing rates J Neurophysiol. 1972 35 311-325

SCHWINDT P C and W H CALVIN Equivalence of synaptic and injected current in determining the membrane potential trajectory during motoneuron rhythmic firing Brain Res 1973 a 59 389-394

SCHWINDT P C and W H CALVIN Nature of conductances underlying rhythmic firing in cat spinal motoneurons J Neurophysiol 1973 36 955-973

SHAPIRO B I and F K LENHERR, Hodgkin-Huxley axon. Increased modulation and linearity of response to constant current stimulus Biophysical Journal 1972 12 1145-1158

SHAPOVALOV A I. Extrapyramidal monosynaptic and disynaptic control of mammalian alpha-motoneurons Brain Res 1972 40 105 116

SHAPOVALOV A I G G KURCHAVYI and M P STROGONOVA, Synaptic mechanisms of vestibulospinal influences on alpha motoneurones Sechenov J Physiol USSR 1966 52 1401-1409

SOKOLOVE P G and I M COOKE Inhibition of impulse activity in a sensory neuron by an electrogenic pump J gen Physiol. 1971 57 125 162

TAKAHASHI, K Slow and fast groups of pyramidal tract cells and their respective membrane properties J Neurophysiol. 1965 28 908 924

TSUKAHARA N K TOYAMA and K KOSAKA, Electrical activity of red nucleus neurones investigated with intracellular microelectrodes Exp Brain Res 1967 4 18 33

WILSON D M. and J L LARIMER, The catch property of ordinary muscle Proc nat. Acad. Sci 1968 61 909-916

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 417

Physical Properties of Connective Tissue as Influenced by Single and Repeated Pregnancies in the Rat

ÅKE RUNDGREN

GÖTEBORG AND ARHUS 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 417

FROM THE DEPARTMENT OF ANATOMY UNIVERSITY OF GÖTEBORG SWEDEN
(HEAD R. EKHOLM)
AND THE DEPARTMENT OF CONNECTIVE TISSUE BIOLOGY INSTITUTE OF ANATOMY
UNIVERSITY OF AARHUS, DENMARK
(HEAD A. VIIDIK)

Physical Properties of Connective Tissue as Influenced by Single and Repeated Pregnancies in the Rat

BY
ÅKE RUNDGREN

GÖTEBORG AND ÅRHUS 1974

Printed in Denmark
by P J Schmidt, V Jens

CONTENTS

GENERAL INTRODUCTION	5
The connective tissue	6
The collagenous framework	6
Ground substance	7
Turnover and catabolism of collagen	8
Geometry of connective tissue	9
Physical properties of collagen	13
Biomechanical properties	14
Basic materials and methods	15
Animals	15
Testing equipment	17
Experimental techniques	19
Parameters	19
Chemical analysis	20
Statistical methods	20
Remarks on experimental methods	20
PART I BIOMECHANICAL PROPERTIES OF THE REPRODUCTIVE TRACT	24
Introduction	24
Uterine horns	25
Uterine cervix	27
Vaginal wall	29
Materials and methods	30
Results	31
Uterine horns	31
Uterine cervix	41

Vaginal wall 50
Discussion 60
Uterine horns 60
Uterine cervix 63
Vaginal wall 67

PART II BIOMECHANICAL PROPERTIES OF EXTRAGENITAL COLLAGENOUS STRUCTURES 70
Introduction 70
Pubic symphysis 70
Posterior cruciate ligament 71
Muscle tendons 72
Skin 72
Materials and methods 74
Results 76
Pubic symphysis 76
Posterior cruciate ligament 80
Muscle tendon 85
Skin 90
Discussion 94
Pubic symphysis 94
Posterior cruciate ligament 96
Muscle tendon 96
Skin 97

PART III THERMAL REACTIVITY IN RAT TAIL TENDON 100
Introduction 100
Materials and methods 102
Results 102
Discussion 107

GENERAL CONCLUSIONS AND SUMMARY 111

ACKNOWLEDGEMENTS 117

REFERENCES 118

APPENDIX 127

GENERAL INTRODUCTION

From the standpoint of physiological function, pregnancy cannot be regarded as a process of fetal growth superimposed on the ordinary metabolism of the mother The development of the fetus is accompanied by extensive changes in the body composition and metabolism of the mother Many of the adjustments begin in early pregnancy before fetal growth is appreciable and therefore cannot be regarded as reactions to "stress" from the growing uterus and its contents.

Much attention has been paid to maternal alterations of the blood volume and composition, cardiovascular dynamics, renal function, alimentary function and water and electrolyte metabolism. On the other hand little interest has been focused on functional aspects of the connective tissues, although they constitute the "skeleton" of all soft tissues. Most studies of connective tissues during pregnancy are done from a biochemical point of view and studies of functional properties have mostly been limited to the genital tract, especially the uterus.

The purpose of the present investigation has been to attempt to at least partially answer the following questions

1 How do the physical properties of the collagenous framework of the reproductive organs change during the different phases of pregnancy and are these changes due to quantitative or qualitative alterations?
2 Are there alterations in the physical properties of the extragenital connective tissue during pregnancy?
3 Are the maternal changes of both the genital and extragenital connective tissue completely reversible or will repeated pregnancies result in altered physical properties compared to virginal animals of the same age?

The connective tissue

Connective tissues contain cells and an extracellular matrix with the latter dominating and consisting of fibers, mostly collagenous but also elastic and reticular and ground substance. The proportions and arrangement of the fibers and the composition of the ground substance vary with the particular type of connective tissue.

The collagenous framework

Collagen the principal fibrous protein on which the physical properties of the connective tissue largely depend, constitutes approximately one third of the total body protein. It is present in phylogenetically very old invertebrate species (Pikkarainen and Kulonen, 1969) and, at least among mammals, exhibits little or no variation between species (Piez and Gross, 1959)

The synthesis of collagen has been reviewed by Udenfriend (1966) Gould (1968) and Rosenbloom and Prockop (1969) A number of mesenchymal cells, not only fibroblasts, osteoblasts and chondroblasts, but also smooth muscle cells (Ross and Klebanoff 1971) can synthesize collagen. The collagen molecule consists of about 330 residues of glycine, 124 of proline, 93 of hydroxyproline and 110 of alanine per 1000 amino acid residues (Pikkarainen, 1968) It is formed from amino acids into three polypeptide chains in the endoplasmic reticulum and is deposited in the extracellular space. In the collagen molecule from tissues such as skin and bone two of the chains are identical and the third differs slightly in amino acid composition. However in collagen from cartilage the molecule is assembled from three identical chains (Strawich and Nimni, 1971) The polypeptide chains are coiled into left hand helices and the molecule itself is a right hand super helix made up of the three polypeptide chains (Rich and Crick, 1955) The entire structure is held together by intramolecular bonds which can be broken by heat or by concentrated electrolyte solutions (Banga et al. 1956 Elden and Boucek, 1962) When the molecular structure is thus broken the individual chains are found as randomly coiled structures. The physical characteristics of the molecular structure have been deduced from physicochemical studies, X ray diffraction analysis and electron microscopy The molecule is a rod-like particle approximately 300 nm in length, 1.5 nm in diameter and has a molecular weight of about 300.000 (Piez, 1967)

The process of fibrogenesis has been reviewed by Wood (1964) and Fitton Jackson (1968). The collagen molecules aggregate into fibrils, but the mechanisms behind this are unknown. Native collagen is formed as cross-striated fibrils of unknown length. The diameter of these fibrils varies depending on the sources of the collagen and the age of the animal (Greenlee and Ross, 1967; Wasserman, 1956). The axial periodicity of the fibril as determined by electron microscopy and small angle X-ray diffraction is 64 nm in the dry state and 68 nm in the wet state (Bear, 1944; Viidik and Ekholm, 1968). The length of the collagen molecule is 4.4 times the native fibril period. It has been suggested that the discrepancy between the native period and molecule length may be explained by a combination of overlaps and gaps between the molecules (Hodge and Petruska, 1962; Olsen, 1963; Smith, 1968). This implies that the cohesive forces act laterally and that there are no end-to-end attachments between the molecules. Collagen fibrils progressively assemble to form fibers 1-12 μm in diameter visible in the ligth microscope. The process of aggregation is accompanied by a change in the solubility of the collagen. Collagen molecules aggregate into fibrils that are soluble in neutral salt solutions. The randomly arranged newly formed fibrils are organized into a more complex fiber meshwork and with time the solubility gradually decreases as a result of increased cross-linking.

The mammalian tendon consists almost entirely of bundles of fibers lying approximately parallel to its long axis. The fibers are presumably as long as the tendon (Bucher, 1962). In more complicated patterns of the collagenous framework other than the tendon, the length of the fiber probably varies considerably.

There is no specific histologic staining method for positive identification of collagen, although there is a correlation between staining properties with for example van Gieson, Mallory and Masson methods and the presence of collagen established by other methods. However, the absence of staining does not exclude the presence of collagen (Harkness, 1961). Birefringence is exhibited in polarized light and is positive in the direction of the longitudinal axis of the fibril.

Ground substance

The collagen fiber framework is closely associated with the amorphous ground

substance which is formed by the same types of cells as collagen molecules and is secreted into the extracellular space. In dense connective tissue such as tendon the amount of ground substance is small, but it is a dominating component in loose connective tissue. This can be shown in electron microscopy using the freeze-etching technique. This method retains the ground substance thereby providing a much better mean of evaluation of this component than is possible in thin sections from plastic embedded tissue. The main components of ground substance are the acid mucopolysaccharides or glucosaminoglycans, the chemistry of which have been reviewed by Muir (1964) Gardell et al. (1969) and Laurent et al. (1969) Most if not all of the mucopolysaccharides in the connective tissue are linked to proteins or peptides by covalent bonds. The mucoproteins and mucopolysaccharide proteins take an integral part in stabilizing the collagen fibers by being firmly bound to the fibrils (Matthews, 1965) However what significance this might have for the rheological parameters in connective tissue is not known (Viidik, 1973) The ground substance is a hydrophilic gel (Chrisman, 1964) which binds water (Ogston, 1966) and the water content influences the physical properties of connective tissue (Galante, 1967)

During aging changes in the composition as well as the concentration of mucopolysaccharides in the connective tissue have been reported. It is probable that these chemical changes are accompanied by changes in the physical properties of the macromolecules which in turn would influence the properties of the tissue (Gardell et al. 1969)

Turnover and catabolism of collagen

The metabolic turnover of collagen is very slow especially in old age (Thompson and Ballou 1956) The liver and bone collagens have the highest rate of turnover while collagen in tendons has a very long half life (Neuberger 1965 Woessner 1968) There are normal and pathologic states of some tissues in which collagen turnover or breakdown is high. Examples of the former are found in the remodelling of bone during growth and the partial resorption or restoration of the uterus after parturition. The mammalian uterus is a very favourable tissue for the study of collagen catabolism under normal physiological conditions because during the course of involution which follows parturition, there is an exceedingly rapid breakdown with little concomitant

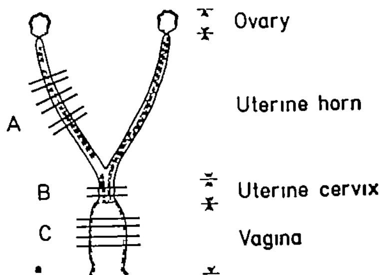
collagen synthesis (Woessner 1962) In a number of bone diseases and hyper thyroidism there are elevated levels of peptide bound hydroxyproline in plasma and urine (Prockop and Kivirikko 1967) In these states and at the particular site involved a collagenase may be present. There are, however only a few sources from which an enzyme can be prepared that will hydrolyze native collagen fibers near pH 7 The concept of collagen breakdown involves tissue collagenase as well as lysosomal enzymes, specifically cathepsin D (Eisen et al. 1970) but it is not clear whether these enzymes attack collagen together at the same site or at independent sites of the molecule.

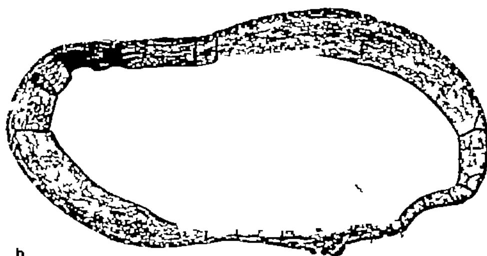
Geometry of connective tissue

The arrangement of the collagenous framework is quite varied and depends on different functional demands. The geometrical pattern of dense connective tissue can be divided into (1) more or less parallel-fibered arrangements, (2) two- and three-dimensional mesh works and (3) complicated patterns where interaction with other elements occur i.e. elastic elements, smooth muscle cells and crystalline solids (Viidik, 1973)

(1) Parallel fibered structures. The tendon is considered to be the most regular parallel-fibered structure. Tendons contain about 30 per cent collagen by wet weight but about 70-80 per cent by dry weight. Elastic and reticular fibers are very scarce. The fibrils are packed closely and parallel to each other making up the tendon fibers which are assembled into fiber bundles. In the relaxed specimen bundles of fibers display a slight waviness (Rigby et al. 1959 Viidik and Ekholm, 1968) The bundles, which are surrounded by sheaths, fuse with each other at acute angles as do the fibers (Viidik, 1973) Fibrocytes are present in long parallel rows between the bundles. A number of bundles are bound by loose connective tissue into large bundles and finally into the complete tendon. Outside the tendon is loose, areolar tissue.

A joint ligament has a similar somewhat less regular parallel-fibered arrangement which is due to differences in functional properties. In ligaments the fiber course is *often partly oblique or spiral. Such geometrical arrangements* have importance for the mechanical behavior of ligaments, i.e. the degree of straining of the various fibers of the ligament varies (Viidik, 1973) Most ligaments consist of pure collagen having elastic fibers only accompanying the blood vessels. A ligament has both its ends inserted into bone, but the


Ovary
Uterine horn
A
B
Uterine cervix
C
Vagina
a


b

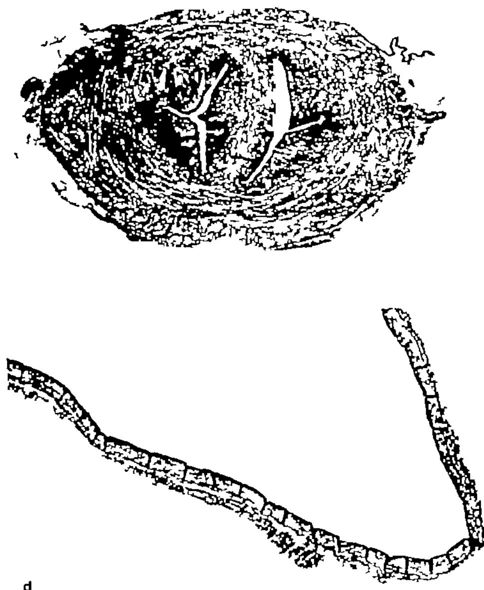


Fig 1 The genital tract in the rat. Schematical picture ( ) with A, B and C indicating the specimen sites. Microphotographs (21 x) of cross-sections of (b) terine horn ( ) uterine cervix (d) and vagina. The specimens were fixated with formalin under intraluminal pressure.

Ovary

Uterine horn

A

B

Uterine cervix

C

Vagina

a

b

fiber arrangement in the symphysis of the rat is mainly an intermingling criss-cross pattern of fibers in the cranio-caudal and dorso-ventral planes. No transversely directed fibers are found (Crelin and Brightman, 1957). Thus the collagenous framework in the pubic symphysis has a complicated three-dimensional structure where the length of the fibers varies considerably. This fact might to some degree explain the observed differences of the load-deformation curve for the pubic symphysis compared to that of the posterior cruciate ligament (Cf. Part II).

Physical properties of collagen

The physical properties of collagen are dependent on the arrangement of the fibers and in particular on the bondings between the components at levels from the molecules to the fibers. Cross-linking of collagen molecules is a normal step in development and is essential for the formation of strong collagen fibers and normal connective tissue (Levene and Gross, 1959. Gross et al. 1963).

When collagen fibers are heated in the presence of water or are exposed to strong solutions of electrolytes they contract (Verzár, 1955). Contraction takes place when the thermal energy is sufficient to break up the ordered structure to a state of random disorder. Covalent cross-links are not affected, only hydrogen bonds and salt-like cross-links are disrupted (Jackson, 1965). In isometric contraction old tissues develop higher tensions than young ones (Verzár, 1963).

Changes in collagen noted with increasing age are considered to be a part of the normal growth and development and are designed to fulfill the need for the bulk of collagen to be insoluble and metabolically inert for optimal function as part of supporting structures (Jackson, 1965). However, in mature fibers Mohanaradhakrishnan and Ramanathan (1965) found that, although an increase of the extent of cross-linking with tanning agents increased the shrinkage temperature, this treatment had the opposite effect on the breaking strength. It would thus seem that there is an optimal number of intermolecular cross-links required to achieve maximal strength, and that an increase in the number of cross-links beyond that point has an adverse effect on the strength.

Biomechanical properties

Mechanical properties of connective tissue are those most clearly related to the function, and information about these can be obtained by biomechanical analyses. A force is required to alter mechanically a specimen of a material. When changes in shape or tension or both are studied the specimen must be fixed to a point at which a counterforce exists that equals the test force. When an object is fixed by one of its ends in a fixture and loaded by means of a weight applied to its mobile end, a tension is created in the specimen. The mass of the weight is measured in grams or kilograms and the tension created between the two ends of the specimen is expressed in newton or in kilopond (the force of 1 kp = 9.81 N = 2.20 lb_f is created when suspending the mass of 1 kg). If the load is large enough the distance between the two ends of the specimen increases resulting in a deformation. This is dependent on the length and cross-sectional area of the specimen. If the material and not a specific specimen with its individual geometrical characteristics is to be investigated, the load is expressed per unit cross-sectional area and is called stress. The original length together with the mechanical properties of the specimen determines how much the specimen is elongated, and therefore the length change is expressed in units of original length and is called strain. If the relationship between stress and strain is linear, i.e., the material is perfectly elastic, this can be expressed as a ratio (e.g. N/mm^2), the modulus of elasticity.

The ultimate tensile strength of a material indicates how much it can be stressed before it fails. There are, however, points before this value is reached which are of interest because no material is perfectly elastic. For soft connective tissues the load-deformation or stress-strain curve (Fig. 4) starts with a toe-part with the concavity towards the load or stress axis. This corresponds to a straightening of the initially wavy formation of the collagenous bundles in tendons (Rigby et al. 1959, Viidik, 1973). With increasing stress the fibers will be arranged in the direction of the load corresponding to a fairly linear section on the curve (Viidik, 1968a). The rupture may occur in this region (Morgan, 1960, Viidik, 1968a) or the curve may bend off towards the deformation axis before rupture occurs. The load value may even decrease below the maximum value before complete failure (Viidik et al. 1965).

The load-deformation and stress-strain curves for collagenous tissues are

sigmoid in shape, and thus the concept modulus of elasticity based on a linear relationship between load and deformation cannot be used. However for the fairly linear part of the curve the tangent value for the angle between the deformation axis and the slope of the linear region can be measured. This is here called "elastic stiffness" for a load-deformation curve and "modulus of elasticity" for a stress-strain curve. These parameters thus indicate the stiffness of the specimen and material respectively for the part of the curve following the toe-part. Many investigators in biomechanics have analysed only the ultimate tensile strength. For collagenous tissues this is reached only when considerable external force is applied. Even then, most often some other part such as the insertion into bone fails before the tendon or the ligament. The analysis should be extended to obtain the abundant information that is offered by the stress-strain curve, especially within the normal functional range of the tissue.

The mechanical properties of connective tissue can be described with a mechanical analogy. It has not been possible to formulate a perfect equation for the visco-elastic behaviour of this tissue because there is a high degree of non-linearity in the load-deformation relationship. Viidik (1968d) proposed a mechanical analogy for parallel fibered collagenous tissue from experiments on knee joint ligaments in rabbits. Based on the analogy a mathematical equation was formulated from which the mechanical properties under certain testing conditions could be predicted, and these were verified experimentally (Frisén et al. 1969a, 1969b).

Basic materials and methods

Animals

Young pregnant rats. 98 female Wistar rats of the same strain, aged 150-170 days, were used. *The estrus cycle was followed by vaginal smears (Fig. 2).* When the smears showed homogenous, nucleated epithelial cells without leucocytes the animals were mated for 12 hours. Then a new smear was made and if spermatozoa could be seen the animal was considered to be in the first day of pregnancy. At the time of testing all non pregnant rats were in the diestrus phase. For the rats that were not sacrificed in the gestational period the time of parturition was recorded and the animals were allowed to nurse



Fig 2 Vaginal smears of a young rat. (a) diestrus phase (b) estrus phase of the estrus cycle (105 x)

their litters. The animals had free access to food and water and were housed under constant conditions of room temperature, air humidity and light.

Old multiparous rats 38 Wistar rats of the same strain as above were investigated at the age of 22 23 months. The animals, except for 9 controls, were mated between the 4-17th months of age. The litters were nursed for three weeks. No pregnancy occurred during the four months before the experiment. A birth record for each rat was carefully kept with regard to date of parturition and size of litter After the fertile period the animals were divided into three groups consisting of those with 4 5 or 6 pregnancies. The animals had free access to food and water and the housing conditions were as noted above. The original number of rats was 58 but during the 17 18 months of observation 20 animals died. During the last four months the number of deaths was seven and of these 3 2 and 2 belonged to the 6 5 and 4 pregnancies groups respectively All rats in the control group survived during the observation time.

Table I summarizes the groups of the young and the old rats, the number of animals and mean body weight for each group.

At the time of the experiment the animals were sacrificed by an overdose of ether and the various organs carefully dissected. The specimens, if not tested immediately were stored in buffered Ringer's solution, pH 7 4 (genital organs) or in saline moisten gauze (pubic symphysis and the intact hind limbs con-

Table I Body weights of the young and old rats used in the present investigation.

	N of animals	g
YOUNG		
Control	16	261 ± 7
Gestational Days		
6–10	3	281 ± 13
13–14	8	298 ± 15**
15–16	9	291 ± 12**
17–18	7	310 ± 12**
19–20	13	318 ± 7**
21	4	329 ± 11**
Post-partum Days		
1	5	281 ± 6**
2	2	270 ± 4
3– 5	8	274 ± 12
7– 9	2	267 ± 4
12–14	7	294 ± 4**
15–16	4	288 ± 8**
17–20	6	287 ± 7**
25–38	4	283 ± 5**
OLD		
N of pregnancies		
0—control	9	315 ± 10**
4	15	359 ± 12FF
5	10	360 ± 18FF
6	4	371 ± 37
4+5+6	29	356 ± 10FF

** 2P<0.05 against young control.
FF 2P<0.05 against old control.

taining the muscle tendons and posterior cruciate ligaments) for a period usually less than two hours at room temperature.

Testing equipment

The materials testing machine is shown in Fig 3 It consists of a horizontal

metal frame with the load transducer (Bofors KRK 1 50 N) at the fixed end. The mobile end was operated via a backlash-free screw arrangement (according to Viidik, 1966) and driven by an electric motor the speed of which was controlled by a tyristor unit. The movement was recorded by a displacement transducer of differential transformer type (Bofors, RLK 1-S ± 6 mm). The clamps to which the specimen was mounted were fastened to the fixed and mobile ends of the system. The deformation speed was kept constant at 1 0 millimeter per minute. Each transducer was coupled to a direct reading measuring bridge (Philips PT 1200) and the resulting load and deformation d.c. signals were fed to the X and Y inputs of a Hewlett Packard Mosely 7004A ink recorder. The clamps with the specimen were placed horizontally in the testing machine (Fig. 3) and balanced by thin steel wires until the specimen, clamps and tranducers were in the same plane. By this procedure influences of vertical forces on the load readings were avoided. The error in the measuring system was estimated for load by multiple loadings of the load cells with known weights and for deformation by displacing the iron core of the differential transformer with the aid of a micrometer. It was in all instances found to be in the range of 0.2 – 0 7 per cent of maximum signals in all measuring ranges.

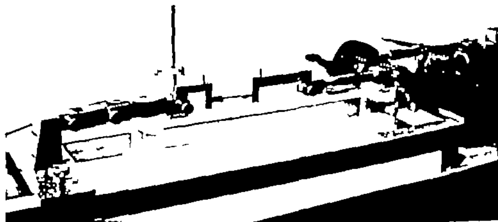


Fig. 3 The materials testing machine. The force transducer is seen to the left. The clamps are in the middle of the picture and to the right the displacement transducer and part of the back-lash free screw arrangement are seen. The motor is situated outside the picture to the right.

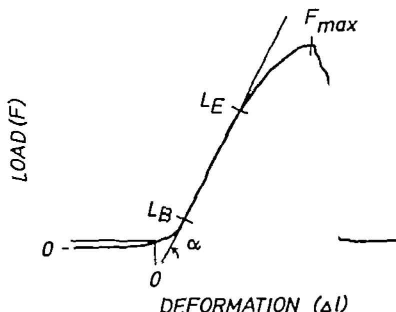


Fig 4 A schematic load-deformation curve for collagenous tissue. L_B = begin of the linear region L_E = end of the linear region F_{max} = point of failure, α = angle between the linear regionand the deformation axis elastic stiffness

Experimental techniques

Parameters

A schematic load-deformation curve for a parallel fibered collagenous tissue is shown in Fig 4 The following parameters were calculated from the curves (1) load (F) and deformation (Δl) for the start of the linear region of the curve, L_B, (2) the end of the same region, L_E, (3) the point of maximum load, F_{max} and (4) the "elastic stiffness expressed as the tangent, tan α, for the angle of the linear region of the curve and the X-axis. The figures of the various parameters were read from the X Y recorder charts to the nearest 0.25 mm. The tan α values were calculated on the basis of the X and Y coordinates of L_B and L_E.

Load and deformation were expressed in newton and millimeter respectively Except for the pubic symphysis and posterior cruciate ligament specimens the load and deformation were also calculated for stress (σ) and strain

(e) For each specimen the load values were normalized to units of collagen (i.e. mg collagen per millimeter specimen length) giving the stress dimension N/mg/mm, and the deformation values were normalized to the original length (l_0) giving the dimensionless strain. By this procedure qualitative differences in the specimens can be analysed. The original length (l_0) is defined here as the length of the specimen when it starts to resist loading, which point was considered to be reached after a two millimeter change in the Y-direction on the recorder chart (at standard calibration for specimens with the highest failure load) corresponding to about 0.08 N

Chemical analysis

After the mechanical test the collagen content in the specimen except for the pubic symphysis and posterior cruciate ligament, was determined by quantitative hydroxyproline analysis according to the method of Stegeman (1958) as modified by Bergman and Loxley (1963) The specimen was hydrolyzed in 6N hydrochloric acid at 130 C for three hours in sealed Pyrex® tubes. Chloramine-T was added to oxidize hydroxyproline to a pyrrole compound which in combination with p-dimethylaminobenzaldehyde yields a red color The red color was measured spectrophotometrically at 568 μm (wave length) The collagen content was calculated as 7 46 times the hydroxyproline content (Neuman and Logan, 1950)

Statistical methods

The load-deformation curves were analysed for the parameters presented above. When more than one specimen from an organ in the same animal was tested, the mean values for the individual animals were used to calculate the mean value of the group For each group mean value and standard error of the mean were calculated and various groups were compared with the control group by Student's t test. Differences were regarded as significant if $2P<0.05$ and tending to be significant if $2P<0.10$ (two-sided test)

Remarks on experimental methods

The techniques used by investigators to evaluate various mechanical properties of connective tissue have varied to a great extent. Most of the testing proce

dures come from the field of materials strength testing, but the methods cannot be adopted without modifications and proper attention to the peculiarities of the biological material. The collagen fiber never functions isolated and is always part of a functional unit as in the locomotive system in a bone-tendon-muscle-tendon-bone or bone-ligament bone complex. Testing the whole complex gives the performance of the collagenous structure to the failure of the weakest point in the system, which never is in the tendon or ligament (Viidik, 1968a) For elucidation of the full range of the properties of the tendon or the ligament it must be tested isolated. There are, however many problems connected with this.

In whole functional units the bones are the natural point for fixation preferably by clamping Testing of isolated tendons creates many problems, especially with regard to adequate clamping Often the results are distorted due to slipping of the specimen or breaking in the jaw of the clamp. Fresh material has low rigidity and when clamped tends to "flow" As will be described in Part II the tendon and skin specimens broke at one of the jaw edges. When the specimen starts to fail, a deviation of the curve towards the strain axis ending the linear region is noted. This point and the breaking point are influenced by the clamping procedure, while the coordinates of the start of the linear region and the elastic stiffness" should be unaffected by this. Jaw breaks were also encountered by Wright and Rennels (1964) in their study of human plantar fascia. They noted that the failure took place somewhere in the linear region of the curves. However conclusions about both the toe-part" and the "modulus of elasticity" could be made.

When evaluating the tensile properties of geometrically non-congruent specimens it is of importance to know the cross-sectional areas. Many difficulties are encountered due, for example to variation in the cross-sectional area throughout the length of the specimen. This and other factors might account for differences in results reported on tensile strength measurements. Tensile strength determinations were well correlated to the values of fresh weight per unit length for tendons from animals of uniform functional state and age (Viidik, 1967) By this technique a measure corresponding to a mean value for the cross-sectional area of the whole tendon was obtained instead of values at arbitrary measuring points. The amount of collagen per unit area or *unit length of the specimen proved to be an adequate parameter* (Fry et al. 1964 Viidik, 1970) This type of measurement was selected for the

assessment of the functional cross-sectional area in the present study because the genital organs and skin have considerable amounts of ground substance and other tissue components in addition to collagen that add to weight but not to strength. The "stress" values are thus load values normalized to mg collagen per mm specimen length. This method was also applied to the muscle tendons since it had been shown to be satisfactory in biomechanical tests of the chordae tendineae (Viidik, 1970)

The other basic parameter to measure is the original length. For parallel fibered collagen specimens it is advantageous to measure the length of the specimen between the clamps after a small but safely recordable stress, corresponding to one to two per cent of the failure value is applied This makes certain that the specimen is straightened in a standardized way When testing a bone-ligament-bone system such as the posterior cruciate ligament of the knee joint and the bone fibrocartilage bone system of the pubic symphysis, the measurement of the original length as well as that of the cross-sectional area becomes almost impossible without damaging the specimen. The insertions of the ligament are fairly broad and the thickness varies being least in the middle. In the pubic symphysis of the rat the width varies along its coronal plane being about one millimeter in the cranial and midsections and somewhat wider caudally The ventral portion of the articular capsule is thick due to the origin of the fibers of the rectus abdominis muscle and obscures the bone-cartilage junction. By disregarding the original length the testing will be qualitative rather than quantitative, but it is fair to assume that the various specimens are reasonable congruent in the same stock of animals of the same age (Viidik et al. 1965 Viidik, 1968c Crelin and Brightman, 1957)

The measurement of the original length in specimens with a lumen such as the genital organs presents another problem. The wall of the specimen in the unloaded conditions is approximately evenly thick. During loading there will be a certain "flow" from the points where the force is applied to the part of the specimen that are not in the line of the force application. The potential source of error for deformation or strain measurements lies in this behaviour of the specimen and will be greater the thicker the wall. Harkness and Harkness (1959a) considers the uterine cervix as a belt and ignored the complications due to the thickness of the wall. They used the inner circumference as the original length measurement after the specimen was loaded for 5 seconds with 0.5 N By applying this load the collagenous framework is

probably streched and the value will not represent the true original length. In the present investigation the ring formed specimen is considered as a solid body. The "flow" of tissue during loading is disregarded as there is no simple method to measure it. The original length of the specimen is measured on a photograph as the distance between the mean of the inner and outer walls at each of the two force application points in the completely unloaded specimen. To this value is added the distance in the recorder chart from the start of the recording to the point on the curve where a change in Y-direction safely has been recorded. This was usually equivalent to about 0.08 N with a little variation due to small changes in the Y-axis calibration.

Testing tendons, ligaments and most other collagenous tissues in completely intravital conditions is impossible. Attention must thus be paid to possible post mortem changes. The water content of the tissue is important because drying increases the stiffness of the tissue (Galante, 1967). Other post mortem changes are less evident. No changes were observed in various tensile strength parameters when storing intact knee joint specimens for as long as 3 days (Viidik et al. 1965. Tipton et al. 1967). This is also in agreement with the results of experiments performed on skin (Ridge and Wright, 1965). For refined rheological analyses it is important to use standardized procedures because post mortem osmotic, chemical and metabolic changes may influence the results (Tipton et al. 1972). Collagenous tissue swells in water (Gustavson, 1956). This is also true for "physiological" saline (Viidik and Lewin 1965) and the tissue exhibits subsequent changes in its mechanical properties after immersion in this solution. The magnitude of swelling in a tissue is also related to its degree of organization and the amount of internal stabilization of its proteins (Gustavson, 1956). A gelatin gel with the same protein content as a piece of skin exhibits much greater swelling than the skin (Galante, 1967). Exposure to air causes progressive water loss from the tissue and is greatest when first exposed. The most effective methods to keep the tissue from drying is to keep it in humidity of 100 per cent (Hirsch and Galante, 1967) or to wrap the structure in a saline moistened gauze (Viidik and Lewin, 1965). The latter technique prevents changes in the mechanical properties for at least 4 hours. By using this technique or immersion in trisbuffered Ringer's solution with a constant pH of 7.4 when necessary the environmental factors are standardized and inter-experiment variation minimized in this series of experiments.

Part I

BIOMECHANICAL PROPERTIES OF THE REPRODUCTIVE TRACT

Introduction

The collagenous part of the connective tissue has a low rate of metabolism in most organs (Neuberger and Slack, 1953) However in the reproductive tract, during a short period of time, the collagenous framework can adapt itself to new functional demands imposed by gestation and parturition and subsequently restore itself in the following involution period. The rapid synthesis and laying down of the different connective tissue components during gestation and the even more rapid post partum breakdown change the functional properties, as have been shown by earlier biomechanical investigations (Harkness, 1964 Harkness & Harkness 1959a, 1961 1965)

A part of the reproductive tract of special interest from a connective tissue point of view is the uterine cervix because of its high collagen content and the extreme requirements placed on its functional behavior at the time of parturition. Biomechanical investigations of the uterine cervix in rat have shown an increased extensibility of the collagenous framework during gestation (De Vaal, 1946 Uyldert and de Vaal, 1947 Harkness and Harkness, 1959a, 1961 Zarrow and Yochim, 1961) The physical properties of the uterine horns have been studied by Harkness and Harkness (1965) who found extensibility unchanged during pregnancy and increased ultimate tensile strength at parturition. Less attention has been devoted to the vagina where the morphological changes during pregnancy are not so conspicuous. Changes in physical properties of the vaginal wall do, however occur (Harkness and Harkness, 1965) but information is available only for a few time intervals during gestation and post partum period.

The purpose of this part of the present investigation is (1) to follow the biomechanical properties of the genital organs during the different phases of a single pregnancy and the subsequent involution, (2) to analyse these

organs for the same properties after repeated pregnancies and (3) to estimate the effect of aging on the physical properties of the genital tract by comparing young and old virginal animals. When analyzing the biomechanical parameters correlation has been made to changes in the collagenous framework.

Uterine horns

Young pregnant rats. A large part of the endometrium in the uterine horns in the rat consist of connective tissue lying beneath the epithlium. The endometrial collagen fiber mesh work in virginal rats consists mostly of coarse bundles but during pregnancy the larger fiber bundles are transformed into a network of small anastomosing fibers and the amount of ground substance increases (Fainstat, 1961) The myometrium has one inner circular and one outer longitudinal layer of smooth muscle fibers separated by scant amount of connective tissue. Just above the uterine cervix the smooth muscle cells constitute 40-50 per cent of the total tissue volume of the non-pregnant rat as estimated by histological methods (Harkness and Harkness, 1959a)

The estrus cycle changes the wet weight of the uterus considerably with a maximum of 128 per cent at estrus and a minimum of 84 per cent at diestrus based on the mean values for all stages but does not influence its collagen content (Harkness et al. 1957) The collagen concentration in the horns of non-pregnant rats at estrus is 2.8 per cent of the wet weight and decreases to 2.2 per cent at the end of gestation (Harkness and Harkness, 1954) Of the total protein content of rat uterus the collagen constitutes 30 per cent in the non-pregnant state and 20 per cent during late gestation (Montfort and Pérez Tamayo, 1961)

During gestation in the rat the wet weight of the uterus increases about seven times and the total collagen content six times, but the increase in both these parameters is slight until the 12th day of gestation, when a rapid growth ensues (Harkness and Harkness, 1954) During gestation in an empty horn secondary to ovariectomy on one side, some growth of collagen takes place in the first half of pregnancy (Harkness and Harkness, 1956 Harkness and Moralee, 1956) While the horn containing fetuses continues to grow the empty horn remains unchanged for the rest of the gestation. There is also evidence that distension of the uterine horn with wax increases the amount of collagen (Cullen and Harkness, 1959) After parturition the wet weight

and collagen content decrease to a level about 50 per cent below the values of virginal animals of equal age (Harkness and Moralee, 1956 Woessner and Brewer 1963) During the early post partum period abundant macrophages *with conspicuous enzymatic activity probably take part in the degradation of* ground substance and fibrillar elements (Lobel and Deane, 1961) and fragments of collagen fibrils have been found by electron microscopy in these cells (Brandes and Anton, 1969) Increased concentrations of proteolytic enzymes in the uterus during the involution have been reported (Woessner 1962 1968 Woessner and Brewer 1963 Schaub 1965/66) Jeffrey and Gross (1970) were able to partly isolate and characterize a collagenase from involuting horns and Ryan and Woessner (1971) demonstrated collagenase activity from similar tissue.

Reports concerning elastin in the uterus are much more scarce than about collagen. Histological investigations have shown that most of the elastin is in the walls of the blood vessels, although elastic fibers can be seen throughout the myometrium (Albert, 1966) Chemical studies on human uterus indicate that during pregnancy the amount of elastin increases proportionally as much as collagen about five to six times and decreases during the post partum period (Woessner and Brewer 1963)

The ground substance of the uterus has mostly been studied by histological techniques. As judged by these investigations there is an increase in the ground substance of the uterine connective tissue in rat during gestation (Maibenco 1960)

Investigations of physical properties of the uterus have been concerned more often with the smooth muscle cells than the connective tissue (Scapo and Goodall 1954 Zimmer 1959) Harkness and Harkness (1965) determined the ultimate tensile strength and "extensibility" (i.e rate of specimen extension at a constant load) in ringshaped specimens from uterine horns from virginal gestational and one day post partum rats. The tensile strength expressed in N/mm² (cross-section area calculated to be pure collagen) was 13 on the 12th day of pregnancy and reached 29 on the 21st day of pregnancy 24 hours post partum this value was reduced to 12, approximately the same as that in virginal animals. There was no evidence of change in extensibility" at parturition while the uterine cervix showed very high values for this property at this time.

Old multiparous rats Morphologic studies on the effect of repeated preg

nancies in intact and unilaterally ovariectomized mice have been performed by Biggers et al. (1962) who found the functioning uterine horn in the operated animals hypertrophied. This shows the importance of mechanical distension caused by the fetuses as a growth stimulus since the hormonal influence was the same for both the active and inactive horn. Finn et al. (1963) repeated the investigation and determined the collagen content in the horns of mice at the age of 24 months. They found the difference between the two horns negligible. No old virginal animals were, however included in the study Compared with two month old virginal animals the collagen content in the uterine horns had increased three times. The increase in the collagen content of whole uterus in virginal rats is negligible after early adult life (McGavack and Kao, 1963) Maurer and Foote (1972) reported that three to four days after parturition in multiparous rabbits of different age and number of previous pregnancies, the uterine collagen content tended to increase and the specific collagenase activity decreased with the number of pregnancies. No virginal animals were included in the investigation. Burack et al. (1941) studied by light microscopy the connective tissue in the reproductive tract of rats from young to old age. In virginal animals the growth of connective tissue was greater than in those with previous pregnancies and contributed to an increased thickness of the wall of 30 per cent compared to highly fertile breeders. In a biochemical investigation on biopsy materials from multiparous women Woessner and Brewer (1963) found an increase in the wet weight, the amount of collagen and the amount of elastin in the uterus compared to virginal females.

Uterine cervix

Young pregnant rats The uterine cervix of the rat, compared to other parts of the reproductive tract is a rather compact structure that can be easily distinguished although its transition to the uterine horns is somewhat gradual (Harkness and Harkness, 1959a) Histologically the conective tissue represents 50–65 per cent of all the tissue in the cervix. The smooth muscle cells are sparsely represented in the portio but increase cranially (Harkness and Harkness, 1959a)

The wet weight of the cervix in the rat varies with the estrus cycle but the dry weight does not (Datta et al. 1968) During gestation the increase in wet

weight starts after the first half of gestation. The increase of collagen parallels in time that of the wet weight being about doubled at parturition (Harkness and Harkness, 1959) At the time of parturition hexosamine is 2.5 per cent of dry weight compared to non pregnant rats, which have 0.5 per cent. The walls of the cervix are edematous and the bundles of collagen fibers are separated from each other by the abundant ground substance (Storey 1957 Leppi, 1964 Bryant et al. 1968 Leppi and Kinnison, 1971)

During the post partum phase the wet weight is reduced approximately eight times and the collagen content four to five times (Harkness and Harkness, 1961) While the wet weight reduction is noted almost immediately after parturition the catabolism of collagen is delayed for about 48 hours (Harkness and Harkness, 1959) Temperature variations as well as papaverine in were discussed above in connection with the uterine horns.

The ultimate tensile strength of the uterine cervix in the rat expressed in N/mm² collagen, is 13 2 at estrus and 9 8 at the ?1st day of gestation (Harkness and Harkness, 1959a) Temperature variations as well as papaverine in the medium in which mechanical tests were performed do not influence the ultimate tensile strength except at parturition. At this time lower values were reported at 37 C than at 22 C. During parturition tensile strength measured at 22 C is 11 9 On the second day post partum it was 9.9 and on the 8th and 16th day 8.4 (Harkness and Harkness, 1961) The load-deformation procedure used by Harkness and Harkness (1959a, 1961) was a stepwise increase of load until the breaking point. Every 15 seconds a 0.25 N load was added, and before every increase of load the specimen was unloaded momentarily and the force on the tissue reduced to about zero.

The "extensibility or deformation per unit of time of the uterine cervix during pregnancy was analysed by Harkness and Harkness (1959a, 1961) When studying this "creep" phenomenon the specimen was subjected to constant load for different periods of time. The cervix usually yielded rapidly in the beginning, but with time the specimen became more unyielding and the recorded curve reached an asymptote. At the time of parturition the ex tensibility" was much more pronounced than during the rest of the pregnancy This increased "extensibility" disappeared during the first day of the post partum period and values in the range of the control animals were recorded.

Old multiparous rats. Burack et al. (1941) studied by light microscopy the connective tissue of the uterine cervix in both virginal and multiparous rats

from young to old age. In virgins the growth of connective tissue was greater than in breeding animals and resulted in decidedly larger cervices compared to breeders of the same age, but no actual measurements were reported. By the age of 20 months the stroma of the uterine cervix in virginal mice had become markedly fibrous or hyaline (Loeb et al. 1939) No information on biomechanical properties of uterine cervix in multiparous animals is known to the author

Vaginal wall

Young pregnant rats. The vaginal wall in the rat is a highly collagenous structure with a collagen concentration of about 8 per cent of the wet weight in the non-pregnant animals during estrus (Harkness and Harkness, 1965) The histological picture of the connective tissue in the vaginal wall of young virginal rats is usually that of a closely interwoven collagenous meshwork in which a considerable number of fibroblasts are embedded and in which there is no distinct arrangement of the fibers (Burack et al. 1941)

Morphological changes in the vaginal wall of the rat during pregnancy have not been as thoroughly investigated as in man. Riehm (1951) reported that in the non-pregnant human vaginal wall the connective tissue is of the loose fibrillary type and contains fairly numerous elastic fibers. This type of connective tissue is found in many places in the body During gestation the connective tissue becomes looser due to increased amounts of ground substance as judged by histological methods. The larger collagen fibers found in non-pregnant individuals are not seen and instead small delicate fibers are identified.

Harkness and Harkness (1954) measured the collagen content and the wet weight during pregnancy in the rat. The weight increase about 50 per cent to the time of parturition, and during the involution phase it decreases towards the non-pregnant values. The total amount of collagen in the vaginal wall does not change during pregnancy but the collagen concentration decreases due to the weight increase. During gestation the circumference of the vaginal wall increases about 100 per cent to 50 millimeters compared to non-pregnant diestrus rats. The circumference at parturition is larger than the circumference of the fetal head which is reported to be about 37 millimeters (Harkness and Harkness, 1959a) One day after parturition this value

g
Partus
4
3
2
1
Control
5 10 15 20 1 5 10 15 20 25 30
Days of gestation
Days of post-partum

Fig 6 Wet weight of the uterine horns of young rats during pregnancy (Mean values ± S.E.M.)

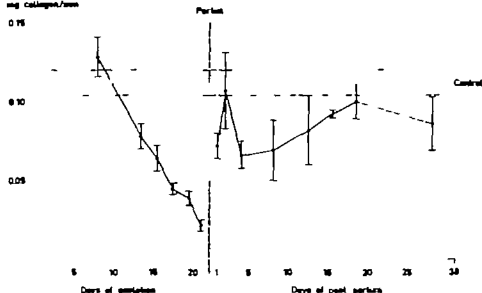


Fig 7 Amount of collagen per unit specimen length of the uterine horns of young rats during pregnancy (Mean values ± S.E.M.)

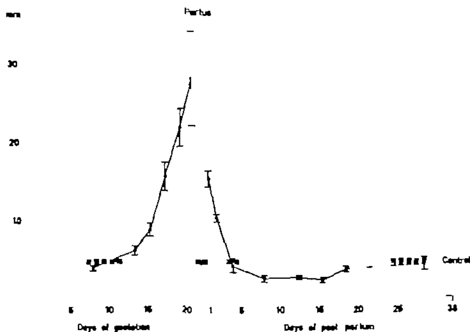


Fig 8 Original length (l_0) of uterine horns of young rats during pregnancy (Mean values ± S.E.M.)

fifth of the value of the control group. During the post partum period there is a noticeable increase compared to the animals of late gestation. Due to rapid wet weight reduction and delayed collagen catabolism during the first days after parturition, the amount of collagen increases but then a slight decrease occurs. During late post partum there is again an increase towards the value of the control group.

The original lengths (l_0 = the length of the specimens when they start to resist force) are considerably increased during gestation (Fig 8) reflecting the distension of the uterine wall by the growing fetuses. The involution progresses rapidly during the early post partum period and after the fifth day the original length is less than in the virginal animals. In the late post partum period the values approach those of the controls again.

During the mechanical testings the specimens usually failed at the end near and lateral to the point where the clamp was in contact with the tissue. The results of the gestational and post partum uterine horn specimens from these

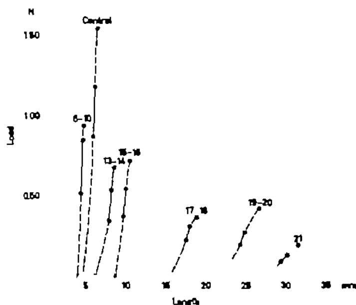


Fig 9 Mean load-length curves of uterine born specimens of young rats during gestation. The figures of the tops of the curves denote days of gestation.

Fig 10 Mean load-length curves of uterine born specimens of young rats during the post partum period. The figures of the tops of the curves denote days after parturition.

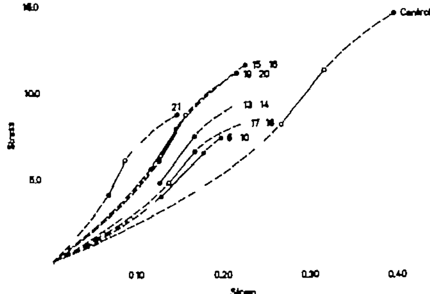


Fig. 11 Mean stress-strain curves of uterine horn specimens of young rats during gestation. The figures at the tops of the curves denote days of gestation.

tests are presented in Figs. 9 and 10 and Table A I (A = appendix). The load-deformation values are listed and curves for load versus length for the different groups show the relaxed length at zero load, after which the actual load-deformation curve begins. Due to the growth of the horns during gestation the curves move on the length-deformation axis towards larger values. There is also an increase in the total deformation of the specimen, 1.9 mm for the control and 4.8 mm for the 19-20 day group. The maximum load is by far greatest for the control group and during gestation shows a sharp reduction approaching parturition. The breaking load for the 21 day animals is only approximately 15 per cent compared to the controls. In the linear region of the curves the "elastic stiffness" also shows a decrease during gestation. Deformation per unit load increases considerably and is most conspicuous near parturition.

During the first two days post partum the load values increase but they are sharply reduced when catabolism of the collagen starts. The reduction is

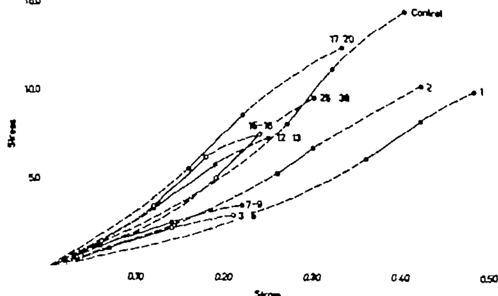


Fig. 12 Mean stress-strain curves of uterine horn specimens of young rats during the post partum period. The figures at the tops of the curves denote days after parturition.

maintained until about 12 13 days at which time an increase occurs and continues until the late post partum period. During the early phase the defor mation of the specimens is pronounced. During the first day the elongation at the breaking point is 7.5 mm, for the second day 4 4 mm and at 15-16 days this value is only 0.6 mm. The "elastic stiffness is low during the early post partum period but increases after the 12th day towards the control value, but this is not reached during the time period studied.

The stress-strain relationships are shown in Figs. 11 and 12 Table A II. During gestation the strain is reduced for the coordinates measured. The most pronounced reduction occurs in the 21 day group where the maximum strain is 0 15 compared to the control value of 0 40. The stress is greatest for the control rats and decreases somewhat but not as obviously as for the strain values. For the maximum stress the decrease is least evident, the value for the 19-20 day group being in the range of the control group. With a small reduction in the stress values and a large reduction for the strain, the

Table II Wet weights of the uterine horns (mean values ± S.E.M.) from old rats.

No of pregnancies	No of animals	g
0 = control	9	0.910 ± 0.100
4	14	0.740 ± 0.060
5	10	0.720 ± 0.070
6	4	0.850 ± 0.180
4+5+6	28	0.751 ± 0.044

modulus of elasticity" tends to increase near parturition, although the differences are not significant compared to the control group. From the appearance of the curves the post partum period can be divided into different phases. The first and second day groups show increases in maximum strain and decreases in maximum stress compared to the control, but compared to the late gestational groups the stress is not altered but the strain is. From the 3rd to the 9th day when the collagenous framework is broken down, stress and strain values are very low indicating a loss of the force resisting properties. From the 12th day and onward stress values increase and the 17 20 day group almost reaches the control group values. During the latter half of the post partum period there is thus a restoration of the biomechanical properties. This phenomenon is also seen in the values for the elastic stiffness.

Old multiparous rats The fresh weights of the horns (Table II) in the multiparous groups all tend to have lower weights compared to the virgins although the differences are not significant ($2P < 0.05$)

The amount of collagen per unit specimen length (Table III) for the

Table III Amount of collagen per unit specimen length of uterine horn specimens (mean values ± S.E.M.) from old rats.

No of pregnancies	No of animals	mg/mm
0 = control	9	0.2547 ± 0.0266
4	14	0.1508 ± 0.0115
5	10	0.1107 ± 0.0092*
6	4	0.1755 ± 0.0228**
4+5+6	28	0.1400 ± 0.0084**

$P < 0.05$ against control.

Table IV The original length (l_0) f uterine horn specimens (mean values ± S.E.M.) from old ats

No of pregnancies	No of animals	
0=control	9	5.2 ± 0.2
4	14	5.0 ± 0.3
5	10	5.5 ± 0.5
6	4	5.0 ± 0.8
4+5+6	28	5.2 ± 0.5

multiparous animals in the different groups is significantly higher than that of the control group. The original lengths of the specimens (Table IV) do not differ very much between the groups. The pooled group of all multiparous animals has actually the same mean value as the control group.

The biomechanical properties of the uterine horn specimens given in

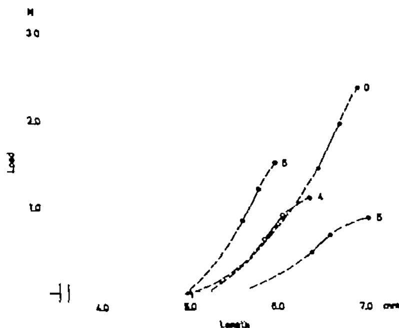


Fig 13 Mean load-length curves of uterine horn specimens of old multiparous rats. The figures at the tops of the curves denote number of pregnancies.

nominal values are presented in Fig 13 and Table A I. The deformation values for the multiparous animals compared to the virginal ones does not differ significantly in any of the coordinates measured. The lowest values are found in the "4" and "6" pregnancies groups. Except for the "6" pregnancies group the load is significantly lower for the coordinates measured. This is in accordance with the amount of collagen per unit specimen length which was highest in the control group. The "elastic stiffness" parallels the load readings with low values among the multiparous animals. The stress-strain relationship, which is presented in Fig 14 and Table A II shows that the strain is significantly reduced at the start and end of the linear region for the multiparous animals. For the maximum strain only the "6" pregnancies group is significantly reduced compared to the virgins. The stress values for the control group tend to be higher than for the multiparous animals but the differences are not significant. Also the values for the "modulus of elasticity" show only small variations.

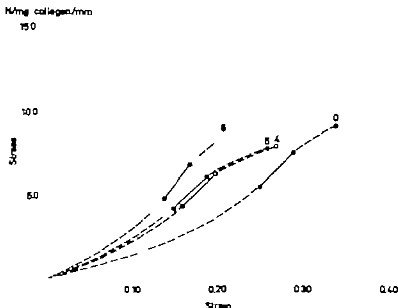


Fig 14 Mean stress-strain curves of uterine horn specimens of old multiparous rats. The figures at the tops of the curves denote number of pregnancies.

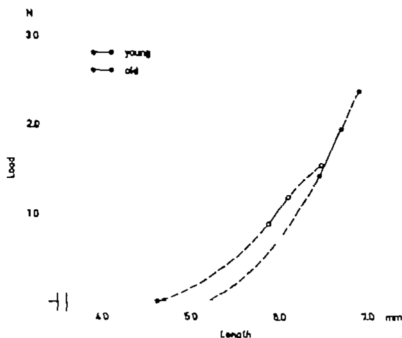


Fig. 15 Mean load-length curves of uterine horn specimens of young and old virginal rats.

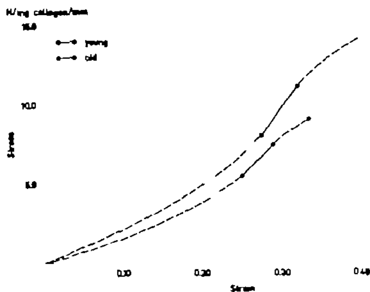


Fig. 16 Mean stress-strain curves of uterine horn specimens of young and old virginal rats.

Young and old virginal rats The biomechanical properties for these animals are compared in Figs. 15 and 16 and Table A I and A II. The load-deformation relationship shows only slight differences with higher values for the old animals. Compared to the young rats the original length has increased from 4 6 to 5.2 mm in the old ones. The amount of collagen per mm specimen length shows a significant increase of more than 100 per cent in the old animals. These figures explain the higher load-deformation values for the old animals.

The stress-strain relationship however shows higher values for the young animals, and the stress values for the end of the linear region and for the breaking point are significantly different from the old rats. Also the modulus of elasticity is decreased in the old virgins.

Uterine cervix

Young pregnant rats The wet weight of the cervix (Fig 17) increases approximately 100 per cent during gestation and decreases after parturition reaching about 50 per cent below the value of the non pregnant animals. During the late post-partum period the weight increases again and is in the range of the control animals. The amount of collagen per unit length of the specimen, shown in Fig 18 decreases during gestation with about 50 per cent. The values during the post partum phase vary to some extent. In the early post partum period the values are much greater than those found in late gestation and also greater than for the non pregnant animals. Then the value tends to decrease and in the late post partum period it is similar to that found in the non pregnant rat.

The original length (Fig 19) starts to increase at approximately the 13-14 day of gestation, and reaches its highest value just before parturition. During the involution period the cervical diameter decreases noticeably the first day after parturition and then somewhat more slowly During the late post partum phase the values reaches those of the control animals.

The load length values are given in Figs. 20 21 and 22 and those for load-deformation in Table A III. During gestation the start of the curve moves along the length axis due to the increase in the circumference of the original length. There is also an increase in the deformation of the curve. The maximum deformation at the rupture point is 1.9 mm in non-pregnant animals

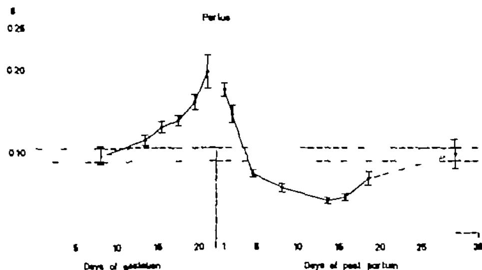


Fig 17 Wet weight of the uterine cervix of young rats during pregnancy (Mean values ± S.E.M.)

mg collagen/mm
Partus
0.75
0.50
0.25
Control
5
10
15
20
1
5
10
15
20
25
30
Days of gestation
Days of post partum

Fig 18 Amount of collagen per unit specimen length of uterine cervix specimens of young rats during pregnancy (Mean values ± S.E.M.)

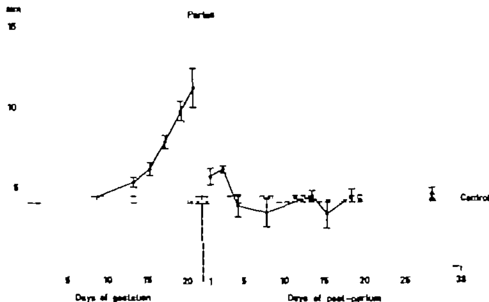


Fig 19 Original length (l_0) of uterine cervix specimens of young rats during pregnancy (Mean values ± S.E.M.)

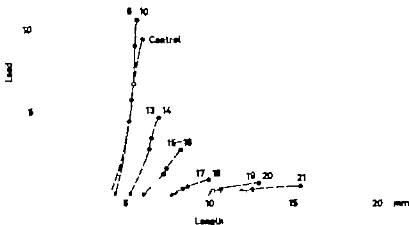


Fig 20 Mean load-length curves of uterine cervix specimens of young rats during gestation. The figures at the tops f the curves denote days of gestation.

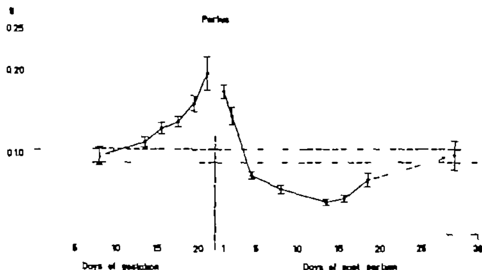


Fig 17 Wet weight of the uterine cervix of young rats during pregnancy (Mean values ± S.E.M.)

mg collagen/mm
Partus
0.75
0.50
0.25
Control
5 10 15 20 1 5 10 15 20 25 30
Days of gestation
Days of post-partum

Fig 18 Amount of collagen per unit specimen length of uterine cervix specimens of young rats during pregnancy (Mean values ± S.E.M.)

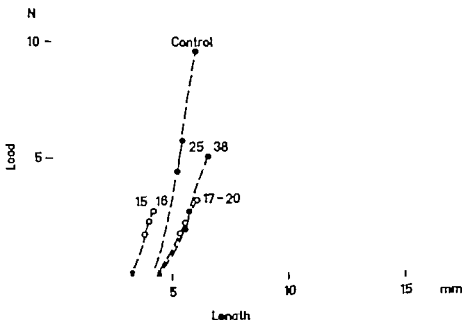


Fig 22 Mean load-length curves of uterine cervix specimens of young rats during the late post-partum period. The figures at the tops of the curves denote days after parturition.

significant increase on the first day compared to late gestation. Then there is a pronounced decrease in this value and although the values again rise in the late post partum phase they do not reach the virginal level within the period of observation. The "elastic stiffness" follows the variation in the load values as compared to late gestation. The value increases on the first day but then decreases when the collagen is broken down. During the late post partum period the "elastic stiffness" again increases.

The stress-strain relationships are presented in Figs. 23, 24 and 25 and Table A IV. During gestation there is a decrease of the strain values most pronounced during late gestation. The stress values decrease during gestation with about 90 per cent for all the coordinates measured. At the time of parturition the force resisting properties of the collagenous framework is reduced. This is, however, not the case for the cervices in the group at one day post-partum. The mean curve for this group has a much more pronounced toe

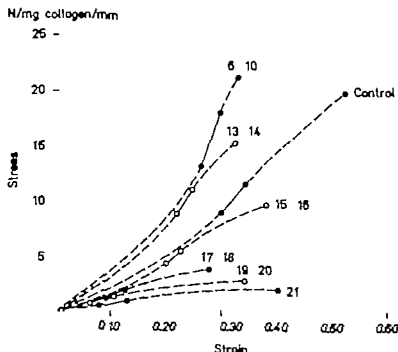


Fig 23 Mean stress-strain curves of uterine cervix specimens of young rats during pregnancy The figures at the tops of the curves denote days after gestation.

or less resemble those of the control group, although the stress remains somewhat lower The modulus of elasticity" changes in parallel with the stress values with an increase for the last group.

Old multiparous rats. In Table V the wet weights of the whole cervices are given. The differences between the multiparous groups are slight, but in comparison to the virginal animals there is a tendency for an increase, which, however is not significant ($2P<0.05$)

In Table VI the amount of collagen per unit specimen length and in Table VII the original length (l_0) are given. The circumference of the uterine specimens is not influenced by repeated pregnancies, as the values for the virginal group and for all multiparous rats are almost the same. The amount of collagen tends to increase for the "6" pregnancies group while for the other groups no differences are found when compared to the virgins.

In Fig 26 and Table A III *load-deformation values for the multiparous*

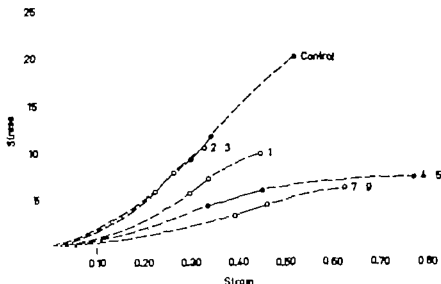


Fig 24 Mean stress-strain curves of uterine cervix specimens of young rats during the early post partum period. The figures at the tops of the curves denote days after parturition

part and higher stress values compared to late gestation. During the period of collagen catabolism there is a larger strain at low stress and the toe-parts are fairly long. During the later part of the post partum period the curves more rats are presented. The mean values for both the multiparous and the pooled virginal animals are fairly similar and there are no significant differences for any of the parameters recorded.

The stress-strain relationships are presented in Fig. 27 and Table IV. Only for the end of the linear region in the 4[th] pregnancies group are the strain values significantly increased compared to the virginal animals, while the strain as well as stress values for the other parameters show no significant differences.

Young and old virginal rats. The biomechanical properties are compared for these animals in Figs. 8 and 29 and Table A III and A IV. In the old animals the original length is increased 60 per cent and the amount of collagen per unit length approximately 100 per cent. The wet weight is also increased

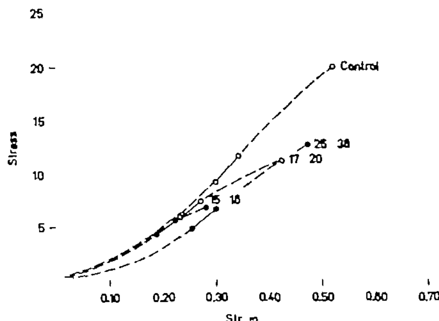


Fig 25 Mean stress-strain curves of terine cervix specimens of young rats during the late post-partum period. The figures at the tops of the curves denote days after par turition

Table V W t weights of the uterin cervix (mean values ± S.E.M) f om old rats.

No of pregnancies	N of animals	g
0=control	6	0 170 ± 0.020
4	10	0.210 ± 0.010
5	9	0.190 ± 0.010
6	4	0.210 ± 0 020
4+5+6	23	0.205 ± 0 008

$2P<0\ 10$ against control.

about 10 per cent. Although there is significantly more collagen in the speci mens from the old animals, the load values compared to the young ones are only slightly but not significantly increased. Due to the increase of the original

Table VI Amount of collagen per unit specimen length of uterine cervix specimens (mean values ± S.E.M.) from old rats.

No of pregnancies	No of animals	mg/mm
0=control	6	0.8100 ± 0.1119
4	10	*1.0118 ± 0.1154*
5	9	0.8800 ± 0.0966
6	4	1.0397 ± 0.0504
4+5+6	23	0.9651 ± 0.0870

2P<0.10 against control.

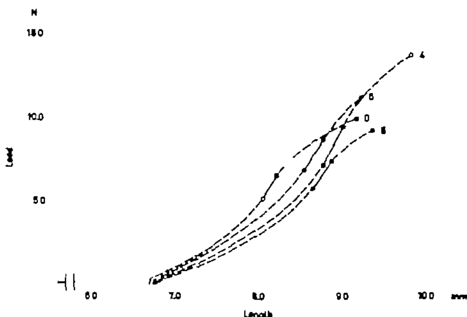


Fig. 26 Mean load-length curves of uterine cervix specimens of old multiparous rats. The figures at the tops of the curves denote number of pregnancies.

length the deformation values of the specimens in the old group is significantly greater The "elastic stiffness" is the same in both groups.

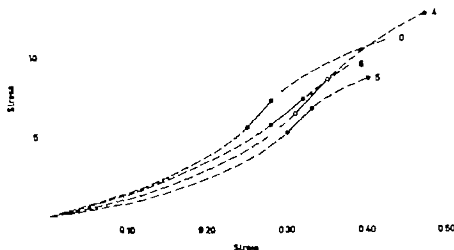
Both strain and the "modulus of elasticity" values are the same in young and old rats, but the stress values are significantly lower in the old group.

Table VII Original length (l_0) of uterine ervix pecimens (mean values ± S.E.M.) f om old rats.

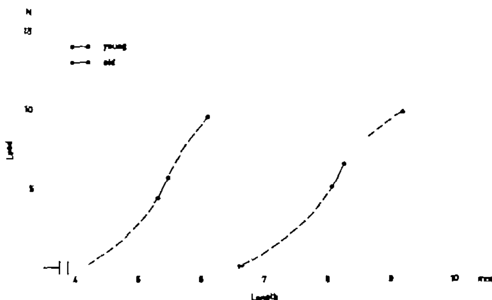
N of pregnancies	No of animals	mm
0=control	6	6 6 ± 0.3
4	10	6.7 ± 0.2
5	9	6.8 ± 0.3
6	4	6 7 ± 0.6
4+5+6	23	6 7 ± 0 2

Vaginal wall

Young pregnant rats The wet weight of the vagina (Fig 30) is increased during the first half of the gestation and the maximum value is reached just before parturition. As with other parts of the genital tract, there is a weight redution during the first half of the post partum phase, which, however is not so pronounced compared to the uterine horns and cervix. A



F g 7 Mean stress-strain curves of uterine cervix specimens of ld multiparous rats. The figures t the tops of the curves denote number of pregnancies



F g 28 Mean load-length curves of terine cervix specimens of young and old virginal rats.

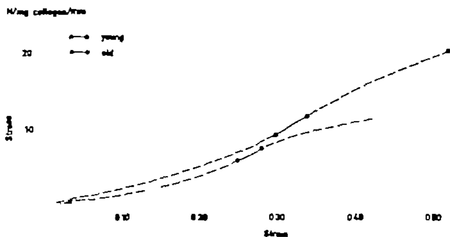


Fig 29 Mean stress-strain curves of terine cervix specimens of young and old virginal rats.

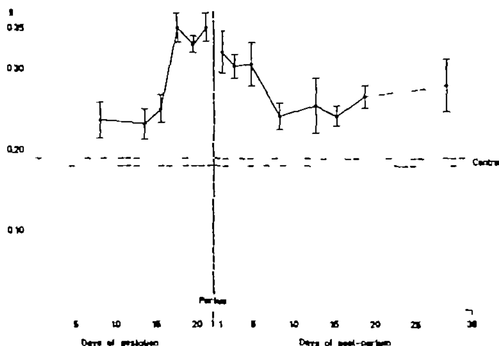


Fig 30 Wet weight of the vagina of young rats during pregnancy (Mean values ± S.E.M.)

weight increase seems to persist at the end of the post-partum period studied in comparison to the non-pregnant animals. The amount of collagen per unit length (Fig 31) decreases during gestation by a little less than 50 per cent towards parturition. During the involution period higher values than at the end of gestation are noted for the first week followed by a decrease, and in the late post partum the values approach those of the non pregnant state.

The variation of the original length during pregnancy is shown in Fig 32 The increase during gestation is notable in the 6-10 day group and the maximum value is reached around parturition with a 60 per cent increase compared to the controls. After parturition the original length decreases and during the late post partum period approaches the values of non pregnant rats.

The load length relationships for the vaginal wall during the gestation and post partum periods are shown in Figs. 33 and 34 and the load-deformation

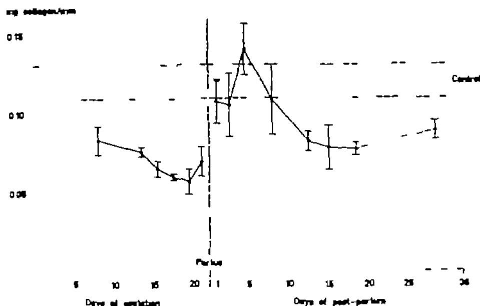


Fig. 31 The amount of collagen per unit specimen length of vaginal wall specimens of young rats during pregnancy (Mean values ± S.E.M.)

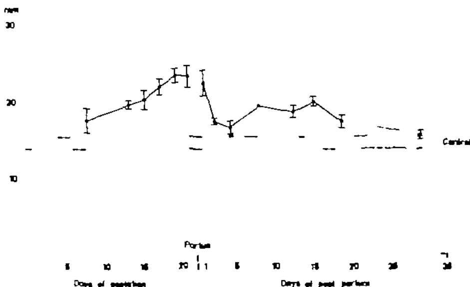


Fig. 32 Original length (l_0) of vaginal wall specimens of young rats during pregnancy (Mean alues ± S.E.M.)

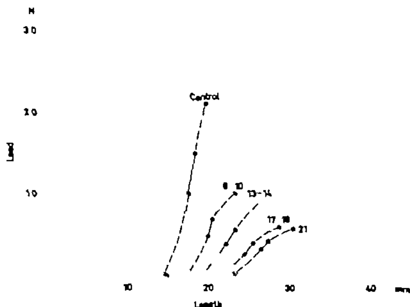


Fig 33 Mean load-length curves of vaginal wall specimens of young rats during gestation. The figures at the tops of the curves denote days of gestation.

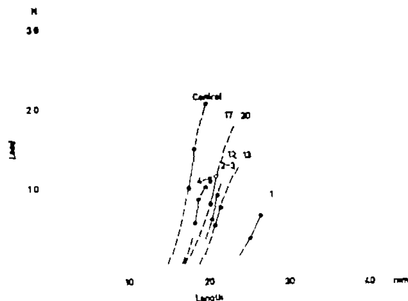


Fig 34 Mean load-length curves of vaginal wall specimens of young rats during the post-partum period. The figures at the tops of the curves denote days after parturition

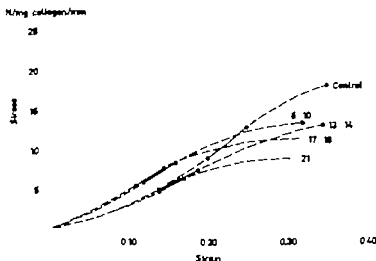


Fig 35 Mean stress-strain curves of vaginal wall specimens of young rats during gestation. The figures t the tops of the curves denote days of gestation.

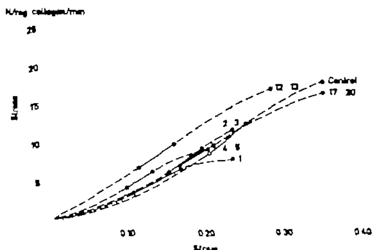


Fig 36 Mean stress-strain curves of vaginal wall specimens of young rats during the post-partum period The figures t the tops of the curves denote days after parturition

parameters in Table A V The deformation of the specimens at failure increases during gestation. In non-pregnant animals this value is 4.9 mm, in the 15-16 day group 6.0 mm and at the 21st day 7 1 mm. The breaking load decreases more than 3 times to the time of parturition. This reduction is most pronounced in the first half of gestation. There is a decrease in elastic stiffness from 0 7 N/mm in non-pregnant animals to 0 ? N/mm just before parturition. During the involution period the maximum deformation is slightly reduced and the maximum load is increased. The "elastic stiffness" parallels the changes in the load readings showing increasing values during the post partum phase. During the late post partum period the values are close to the non-pregnant level.

The corresponding stress-strain values are presented in Figs. 35 and 36 and Table A VI Throughout gestation the strain is with a few exceptions lower for the start and end of the linear region. The toe-part is less pronounced in gestational than in non pregnant animals. The stress values are, with the exception of the 6-10 and 19-20 day group lower during gestation compared to the control. The ultimate stress at the 21st day is less than half the value for the non pregnant animals. The modulus of elasticity" is, however only decreased during gestation for the 13-14 day group

The stress-strain curve at one day post partum is very similar to the curve for the 21st day of gestation. The strain values remain generally significantly lower during the post partum period than the controls but increase in the late phase of involution. The stress values are also lower but beginning from about one week they gradually increase and reach the non pregnant level about the 14-15th day The "modulus of elasticity is significantly decreased

Table VIII W t weights f the vagina (mean values ± S.E.M) from old ats.

N of pregnancies	N of animals	g
0—control	9	0.297 ± 0 033
4	15	0.387 ± 0.015**
5	9	0 402 ± 0.029**
6	4	0 383 ± 0.049
4+5+6	28	0.392 ± 0.013**

** 2P<0.05 against control.

Table IX Amount of collagen per unit specimen length of vaginal wall specimens (mean values ± S.E.M.) from old rats.

No of pregnancies	N of animals	mg/mm
0—Control	9	0.1757 ± 0.0142
4	15	0.1841 ± 0.0127
5	9	0.1677 ± 0.0153
6	4	0.1056 ± 0.0103
4+5+6	28	0.1664 ± 0.0093

P<0.05 against control.

one day after parturition, but otherwise during the post partum period the values are in the range of the controls.

Old multiparous rats The wet weigth of the vagina (Table VIII) is significantly increased in the multiparous animals by about one-third compared to the virgins. For the pooled group of multiparous animals there is no significant difference in the amount of collagen per unit specimen length (Table IX) compared to the virginal rats but the value of the "6 pregnancies group is significantly lower in comparison to the other two multiparous groups and the control group. The virginal group has the shortest original length (Table X) and is significantly lower compared to the 6 pregnancies group.

The load length relationships for the vaginal wall are presented in Fig 37 and the load-deformation parameters in Table A V Since the original length

Table X Original length (l_0) of vaginal wall specimens (mean values ± S.E.M.) from old rats.

N of pregnancies	No of animals	mm
0—control	9	14.6 ± 1.0
4	15	15.6 ± 0.8
5	9	17.2 ± 1.4
6	4	20.5 ± 2.5
4+5+6	28	16.8 ± 0.7

2P<0.10 against control

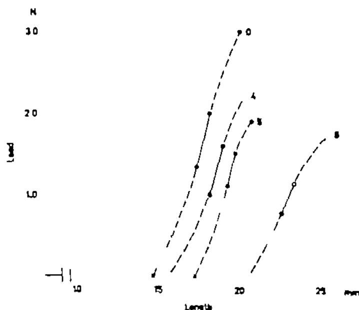


Fig 37 Mean load-length curves of vaginal wall specimens of old multiparous rats. The figures t the tops of the curves denote number of pregnancies.

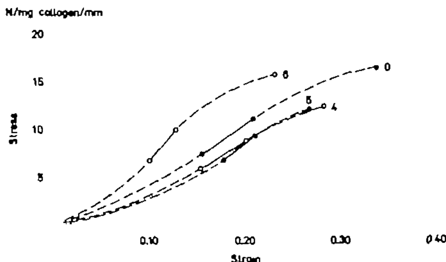


Fig 38 Mean stress-strain curves of vaginal wall specimens of old multiparous rats. The figures at the tops of the curves denote number of pregnancies.

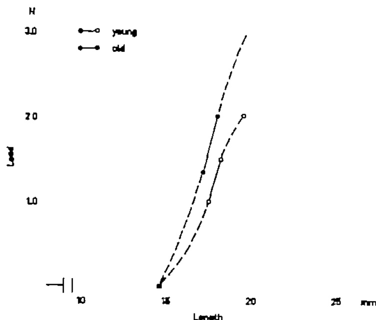


Fig 39 Mean load-length curves of vaginal wall specimens of young and old virginal rats.

increases with the number of pregnancies, the curves for the respective groups move along the length axis. The total deformation does not vary to any noticable degree between the groups, being at most 5.3 mm for the virginal group and at least 3.3 mm in the "5" pregnancies group. The differences between virgins and multiparous animals are more pronounced for the load values where the breaking load is significantly lower for the multiparous groups. The "elastic stiffness" tends also to be lower for the multiparous animals although not significantly different compared to the control group.

The stress-strain values are presented in Fig 38 and Table A VI. The strain values are lower for the "6" pregnancies group than for the other groups. The differences are, however slight when comparing the pooled multiparous animals with the virginal ones. The maximum stress values are significantly reduced in the "4" and "5" pregnancies groups compared to the virgins, but for the other stress parameters no differences are found. The modulus of elasticity is in the same range for all the groups.

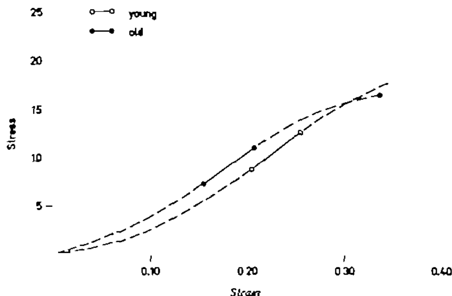


Fig 40 Mean stress-strain curves of vaginal wall specimens of young and old virginal rats.

Young and old virginal rats The load length curves for these animals are compared in Fig 39 and the load-deformation values in Table A V The deformation values are approximately the same in the two groups reaching about 5 mm at the rupture point. The load value for the old rats is significantly higher at the breaking point. There is no difference between the "elastic stiffness" values.

The stress-strain relationship are presented in Fig. 40 and Table A VI The differences between the groups are smaller than for the load-deformation values. Both the stress and strain values are in the same range for both groups.

Discussion

Uterine horns

Young pregnant rats The increase of the wet weight of uterine horns during

gestation (Fig 6) is approximately eight times that found in virginal rats. This is slightly more than was reported by Harkness and Harkness (1954) The decrease of the wet weight during the post partum period is in agreement with other investigations (Harkness and Harkness, 1954 Maibenco 1960) As there is a slower increase in the total amount of collagen than in the wet weight, the collagen concentration decreases (Harkness and Harkness, 1954) In the present experiment the concentration expressed as the amount of collagen per unit specimen length, decreased 4 5 times during gestation (Fig. 7) During the early post partum period the reduction of the wet weight is more rapid than that of the collagen (Harkness and Harkness, 1954) which results in that the amount of collagen per unit specimen length increases compared to the end of gestation although no new collagen is probably formed.

Reports on the decrease of collagen concentration during gestation in the uterine horns of the rat vary somewhat. Harkness and Harkness (1954) found a reduction from 2.6 to 2.2 per cent of wet weight. Monfort and Perez Tamayo (1961) on the other hand also found on wet weight basis a 50 per cent reduction during gestation. In both those investigations the control rats were in the estrus phase. In the present study where the amount of collagen per unit specimen length decreased 4-5 times the control animals were in diestrus phase. The wet weight of the uterine horns is approximately one third lower in diestrus than in estrus but the collagen content is not changed (Harkness et al. 1956) Thus the collagen concentration during the estrus cycle will vary with about 50 per cent. This can partly explain the differences discussed above.

The load-deformation and load-length relationships show the progressive changes of the physical properties of the horns during the pregnancy A reduction of the force resisting properties is very evident at the end of gestation when the *breaking load is reduced 5-6 times compared to the non pregnant* animals. This parallels the decrease in the amount of collagen per unit specimen length which is reduced more than 5 times during gestation. The growth of the uterine horns during gestation results in an increased circumference (Fig. 9) The growth of the wall in the horns is not as rapid as the contents, i.e. the fetuses and placentae. The wall becomes thinner and the amount of collagen per unit area (mg/cm^2) is reduced 5-6 times during gestation (Harkness and Harkness, 1956) From the photographs on which the original lengths were determined, a progressively thinning of the wall during gestation could

tion of the cervix has been a combination of inspection and palpation, which seems satisfactorily reproducible. The increase in wet weight during gestation according to the above mentioned authors is somewhat more than 200 per cent from diestrus values, but in this investigation it is only a little more than 100 per cent. The decrease of the wet weight after parturition is not as pronounced during the first and second day as reported by Zarrow and Yochim (1961) but thereafter the reduction is in agreement with other authors.

Harkness, and Harkness (1959a) found that the inner circumference during *gestation increased almost 400 per cent compared to non-pregnant animals.* In the current investigation the original length increases almost 300 per cent. The collagen concentration during gestation decreased 40 per cent according to Harkness and Harkness (1959a) and according to Zarrow and Yochim (1961) 60 per cent. In the current report the amount of collagen per unit specimen length decreases a little more than 50 per cent. During the post partum phase the collagen concentration increases the first few days because of a more pronounced reduction of other tissues (Harkness and Harkness 1956 Zarrow and Yochim, 1961) (Cf. Fig 18)

These changes indicate that an extensice morphologic rearrangement takes place in the uterine cervix during pregnancy The physical properties are profoundly influenced by these changes. Harkness and Harkness (1959a) found the maximum load for the whole uterine cervix of non pregnant rats to be approximately 900 g, 975 g on the 11 12th day of gestation and about 300 g on the 21st day In the present series the decrease of breaking load is much greater more than 20 times. There is of course a difference in the specimens. Harkness and Harkness (1959a) used whole cervices but in the present study 2 mm wide "ring" specimens are used. This might be of lesser importance when the collagenous framework is more compact as in non pregnant animals but at the end of gestation increased amount of other tissue components such as ground substance (Cf Bryant et al. 1968) separates the collagen fibers. Cutting of fibers in the delicate cervical reticulum might disturb the integrity of the collagenous framework and result in lower values for the load readings. During gestation the cervical specimens show an increase in deformation and a decrease in load. At the end of gestation the total de*formation at rupture is more* than 5 mm at the load of 0 4 N compared to the non pregnant group which has a deformation of 1.9 mm at a load of 9.5 N These changes are also reflected in the more than 40 times decrease in the

"elastic stiffness" values. These changes recorded for the cervical specimens are in accordance with physiological events at parturition. The low loads required for the dilation of the uterine cervix prevent harming of the fetuses. The breaking load according to Harkness and Harkness (1961) was one day post partum 900 g. This is followed by a decrease to approximately 300 g at the 8th day and then at the 16th day a slight increase is noted indicating a restoration of the collagenous framework. These values are similar to those found in the present investigation. During the post partum period the maximum load as well as the other load parameters never reach the low values recorded prior to parturition.

During gestation the decrease in the strain values is most pronounced at the start and end of the linear region of the curves resulting in a diminished toe part of the curve. This can be due to a stretching of the collagenous framework that increases the circumference since other tissue compartments increase more than the collagen content (Harkness and Harkness 1959a). The maximum strain values do not show such a great decrease during gestation. This might be due to an increased amount of ground substance (Bryant et al. 1968) and maybe other changes that allow the fibers to yield or slide in response to mechanical stress above the linear part of the curve.

The stress values during gestation also decrease. The ultimate stress value at the 21st day is 10 times lower compared to the non-pregnant value. There must be qualitative changes or changes in the structural organization in the force resisting properties of the collagenous framework. Harkness and Harkness (1959a) reported that the tension at break in N/mm² collagen for non pregnant diestrus rats is 19 and for the 21st day of gestation, 10. Although the differences in the present investigation are more pronounced partly due to the shape of the specimens, the results are in line with those of Harkness and Harkness (1959a).

Harkness and Harkness (1961) reported that a slight increase of the ultimate tensile strength was noted one day after parturition but Zarrow and Yochim (1961) found this to be 4 times compared to the value at parturition. Such a change is also found in the present investigation. Also the amount of collagen per unit specimen length was remarkably increased one day after parturition but the total amount of collagen was only slightly reduced at this time (Harkness and Harkness, 1961). Bryant et al. (1968) reported that the concentration of hexosamine was 2.51 at parturition and one day later 1.24 g per 100 g

freeze-dried cervical tissue. Thus it might be assumed that there is a condensation of collagen and due to the closer packing a regain of some of its force resisting properties. According to Harkness and Harkness (1961) the "extensibility" which was most pronounced at parturition was completely lost one day later This indicates that the cohesive forces between the collagen units is now stronger than at the time of parturition. During the days following parturition the stress values decrease when the process of collagen break down is most active. Later there is again an increase, a reconstitution of the collagen framework and the stress-strain curves look more like the ones for the controls. The change in the strain values one week post partum results in pronounced toe parts of the stress-strain curves. At this time the collagen catabolism is most active.

Old multiparous rats During the post partum period there is a reconstitution of the uterine cervix towards the non-pregnant state, but the question remains whether repeated pregnancies will permanently change the properties of both the cervix as a whole and its force resisting material per se.

The wet weight of uterine cervix in multiparous animals tends to be somewhat higher compared to virginal animals. This is contrary to what was found in the uterine horns where the controls have the highest value. Harkness and Moralee (1956) showed that the wet weights of both the uterine horns and uterine cervices were reduced almost seven times during the first week of the involution period, reaching values below the non-pregnant level. The original length of the cervical specimens shows hardly any differences while the amount of collagen per millimeter specimen length in the control group has the lowest value. The tendency for the increase of collagen in the mlutiparous rats is not significant compared with the controls. This is again contrary to the findings in the uterine horns where the virgins have a significantly increased amount of collagen compared to the multiparous animals. The loss of collagen from the uterine horns after parturition was, however more pronounced than in the uterine cervix (Harkness and Moralee, 1956) With repeated pregnancies this could result in an increasing amount of collagen in the cervix compared to the horns.

The load-deformation and stress-strain curves are similar between multiparous and virginal animals. The force resisting properties of the collagenous framework thus seem to be almost unaffected by repeated pregnancies.

Young and old virginal rats The load-deformation curves are almost the

same in both groups, although the amount of collagen per unit specimen length and original length are increased in the old animals. The stress values for the old rats are significantly decreased. Although the amount of collagen increases the force resisting properties of the collagen per se in the uterine cervix deteriorates by age. This process is not influenced by multiparousity

Vaginal wall

Young pregnant rats. The changes in the vaginal wall during pregnancy are similar to those in other parts of the reproductive tract although they are less pronounced. The 100 per cent increase in fresh weight during gestation is in good agreement with the findings of Harkness and Harkness (1965) After parturition the weight reduction is less pronounced without hyperinvolution as in the uterine horns and cervix, and the non pregnant values are not reached even in the late post partum period. The original length increases about 60 per cent during gestation and at the 21st day the value of the circumference approximated to twice the original lengh, is 46 mm. This value is in agreement with Harkness and Harkness (1965) However at one day after parturition these authors found for this value a more than 30 per cent decrease compared to late gestation. This is not found in the present investigation. The reduction in the original length at this time is very slight but by the 4th-5th day post partum the reduction is about one-third compared to late gestation.

The amount of collagen per unit specimen length decreases about 50 per cent during gestation compared to diestrus controls. Harkness and Harkness (1965) found the collagen concentration to decrease during the same period about 40 per cent compared to non pregnant rats in diestrus. The increase of collagen during gestation does not keep pace with the total increase in wet weight. Thus other components in the vaginal wall must increase more (i.e. the amount of the ground substance) as was shown histologically in pregnant women (Rechm, 1951) Such changes result in a looser collagenous fiber system. The deformation of the specimen at rupture is increased 1.5 times at the end of the gestation and the load values are reduced 3-4 times. These changes might be a physiological anticipation of parturition allowing a dilatation of the vaginal wall at fairly low forces. After

parturition the deformation decreases somewhat and the load increases without being reduced during the first and second week as in the uterine cervix and horns.

In the stress-strain relationships the strain values are only slightly affected during gestation. The earlier start of the linear region can be due to the adaptability of the collagenous framework to the increased circumference. Since collagen is laid down to a lesser degree than other tissue components, the fibers probably will be less relaxed than in the non pregnant animals. The stress values are reduced during gestation although not so pronounced as for the uterine cervix. The maximum stress at the 21st day is thus about one half the value in the non pregnant rats.

After parturition the stress-strain values remain low for about one week but then there is an increase towards the non-pregnant state. The changes during this period are less pronounced in the vaginal wall than in other parts of the genital tract. The curve for the 17 20 day group is very close to the curve for the control group. The force resisting properties of the collagenous framework of the vaginal wall is reduced during pregnancy It is possible that this reduction might be caused by an increased amount of ground substance that separates the collagenous fibers resulting in a lowered aggregation and a lowered ability to withstand forces. This mechanism might also be responsible for the increase in "extensibility" at the end of gestation that was found by Harkness and Harkness (1965) This "extensibility" is more pronounced on the 12th than 21st day of gestation and returns to non-pregnant values 24 hours after parturition. In the data of the present investigation there is no explanation for the fact that the "extensibility" is greater during mid term than at the time of parturition.

Old multiparous rats After a single pregnancy the reduction of the wet weight during the post-partum period studied does not reach the non-pregnant level. It might thus be assumed that a weight gain will occur with repeated pregnancies which is also the case. All multiparous rats together show no difference with regard to the amount of collagen per unit specimen length compared to the virgins. The weight gain is thus caused by other tissue components than collagen.

The physical properties are also influenced by repeated pregnancies as the maximum load is significantly decreased in the multiparous animals compared to the virgins. In the stress-strain relationships the maximum stress is

significantly lower for the multiparous animals compared to the virgins. The collagenous framework of the vaginal wall thus loses some of its force resisting properties due to repeated pregnancies.

Young and old virginal rats In the old animals the wet weight and the amount of collagen per unit specimen length increase. An increase of collagen in the vaginal wall due to aging was also reported in a histologic investigation of mice by Loeb et al. (1939) In old rats the maximum load value is increased due to the increased amount of collagen but the stress-strain curves are similar between the two groups. The functional properties of the collagen per se as expressed in its force resisting ability are not influenced by aging. Repeated pregnancies seem thus to excert a greater influence on the physical properties, as discussed above, than aging

Part II

BIOMECHANICAL PROPERTIES OF EXTRAGENITAL COLLAGENOUS STRUCTURES

Introduction

Morphologic and functional alternations of the collagenous framework of the reproductive tract as influenced by a single pregnancy repeated pregnancies and aging have been studied fairly extensively (Cf Part I) Harkness (1964) considers it unlikely that the underlying mechanisms are active only locally but rather represent examples of processes of more general character

Relatively little attention has, however been paid to these processes outside the genital tract, with the exception of the pubic symphysis, which at least in some species can be considered a semigenital target organ. The extragenital connective tissues during pregnancy and after repeated pregnancies have been studied only by measuring the isotonic thermal reactivity of rat tail tendons (Cf. Part III)

Pubic symphysis

Young pregnant rats There are some reports in the literature concerning changes of the pubic symphysis during pregnancy (Hisaw and Zarrow 1950 Frieden and Hisaw 1953 Storey 1957 Crelin and Brightman, 1957) As *these changes are most pronounced in mice and guinea pigs*, the reports mainly deal with these species, where alterations during pregnancy involve a complete change of the structure of the tissue.

The cartilagenous symphysis in the non-pregnant mice is replaced by a loose ligament during pregnancy and the distance between the pubic bones increases five times (Storey 1957) The collagen bundles swell and stain more easily with periodic acid-Schiff reagent and silver impregnation methods. Comparison between load-deformation curves for the pubic symphysis from a

non-pregnant and a gestational mouse at the 19th day showed greatly reduced load and increased deformation values for the latter After parturition collagen bundles come closer to each other The ligament is reduced in length and the pubic bones come together There is an increased flexibility in the pubic symphysis of the rat during pregnancy although histologic investigations showed no morphological changes in the joint during this period. Histologically the pubic symphysis in the rat has an intricately arranged three-dimensional collagen fiber net work (Crelin and Brightman 1957)

Old multiparous rats No information concerning changes with aging of the physical properties in the pubic symphysis is available, nor are there any reports concerning the influence of repeated pregnancies. In mice and guinea-pigs the fibrous cartilage is restored after pregnancy and it is not known whether this chronologically younger cartilage also is biologically younger

Posterior cruciate ligament

Knee joint ligaments have been used by several investigators as convenient test specimens. These structures consist of welldefined fairly parallel-fibered collagenous tissue with insertions into the tibia and the femur. The bones or parts of them can easily be fastened without risking slipping or jaw breaks into clamps constructed for material testing machines. Some investigators used the anterior cruciate ligament (Smith, 1954 Viidik et al. 1965 Viidik 1966 1968c) while others have used the collateral ligaments (Schild et al. 1967 Tipton et al. 1967 1969)

It has also been shown that there are no significant differences between right and left legs in measurements of ligamentous strength (Tipton et al. 1967a) Intact ligaments invariably fail at their osseous attachments and variations in breaking force will be those due to changes in the transition ligament nonmineralized fibrocartilage-mineralized fibrocartilage bone (Tipton et al. 1967a) Bone-ligament bone preparations have been used by several investigators for studies on the influence of activity and immobilization on the musculo-skeletal system (Adams, 1966 Viidik, 1968c Tipton et al. 1967b) The physical properties of ligaments under conditions such as pregnancy and aging have attracted little interest.

Muscle tendons

The mechanical behavior of collagenous tissue as found in muscle tendons has been investigated by numerous authors (for review see Viidik, 1966 1973 Galante, 1967) There is good agreement about the form of the load-deformation or stress-strain curve for this type of tissue When testing isolated pieces of parallel fibered collagenous tissue there has always been problems with the fixing of the tissue in clamps without slipping or producing jaw breaks at too low loads. Viidik (1968a) discussed this problem in relation to earlier investigations.

The effect of age changes on the physical properties in muscle tendons have been studied to some extent. Rollhäuser (1950) reported tensile strength values of 30 to 45 N/mm² in newborn against 90 N/mm² in adult humans. Curtis (1962) reported that old tendons have a higher modulus of elasticity than young ones using a slow rate of straining Changes in stress-strain behaviour of tendons were found during growth but not in senescence (Elden, 1969)

During pregnancy Bryant et al. (1968) measured the amount of hydroxyproline and hexosamine in rat tail tendons from six days before to eight days after parturition. During this period of time no changes in either of these parameters were noted.

Repeated pregnancies in rats have been found to influence tail tendons, the collagen of which differs in its physico-chemical properties from collagen in other organs and species. The aging process in all tendons is a continuous process throughout life and lacks the relatively rapid maturation found in collagen from other tissues during the growth period of the life-span (Piez, 1969) It is unknown whether this reflects a lack of function of these specific tendons in the rat or if there are more profound structural differences on the molecular level compared to other collagenous tissues. It is, however not satisfying to rely only upon experiments from tail tendons, and therefore other non-genital connective tissues have been investigated in the present series of experiments.

Skin

Young pregnant rats Müller (1951) studied the microscopic appearance and

the spreading factor of skin during the 16-20th days of gestation in rats in comparison to virginal controls. The dermis was thicker and contained an abundance of coarse collagenous bundles in the pregnant rats. In addition an increased spread of India ink was found.

Bryant et al. (1968) reported the hydroxyproline concentration to be 5 0 per cent in freeze-dried skin from non pregnant rats. This was reduced to *2 7 per cent in pregnant rats six days before parturition* but *increased* to 5 8 at the time of parturition and to 8.7 four days post partum On the eighth day after parturition the hydroxyproline concentration had decreased to 5 6 per cent. The total hexosamine content of skin remained essentially unchanged during pregnancy

The influence of sex hormones on morphological, biochemical and biomechanical parameters of skin has been analysed extensively by Vogel (1970) Small doses of estradiol decreased the maximum load of skin from sexually mature rats during the first and second day of the treatment but larger doses or prolonged treatment with small doses resulted in an increase. Progesterone given in different concentrations increased the maximum load rapidly with only small differences between the groups after the second day

The collagen content of the skin of rats treated with estradiol is reduced as reported by a number of workers (Lobel et al. 1953 Smith and Allison, 1966 Henneman, 1968) although Morgan (1963) was not able to show this. In hamsters the solubility of skin collagen showed no differences between intact, estradiol treated and untreated, prepubertally ovariectomized animals (Kowalevski, 1969) In young intact female rats given 0.5 mg progesterone daily for 14 days the amounts of salt-soluble and acid-soluble collagen increased and the insoluble fraction decreased compared to untreated controls (Holzman et al. 1964)

The ground substance in skin increases after estrogen stimulus due to increase of hyaluronic acid or chondroitin sulphate (Schmidt, 1958) and these effects are subject to species variations (Warren and Fagan 1960)

Old multiparous rats Vogel et al. (1970) found rat skin from both sexes to increase in ultimate tensile strength reaching a plateau with maturity at about 3 months of age. Thereafter a small decrease occured with aging The maximum load did not change much after reaching stable values but the skin thickness increased with the body weight. Fry et al. (1964) reported an increase in breaking strength of approximately three times and a decrease in

"extensibility" (deformation per unit time at constant load) of about 20 times in skin from 20 to 600 days old rats. From the age of three months onwards the collagen concentration did not differ noticeably. Tensile strength increase with age in skin strips was found by Mendoza and Milch (1964/65) when they compared young, 70 days of age, and old, 15 months of age, male and female rats. Significant increase was found for both sexes. Significantly higher values were noted in old male compared to old female rats, but no sex differences were found in the young animals.

The possible correlation between the tensile strength of skin strips from rats of different age and the collagen fractions was analyzed by Vogel (1973). The soluble fractions were found to decrease during aging. Insoluble collagen and total collagen per gram of fresh weight showed a maximum at 4 months of age as did the breaking tensile strength.

Materials and methods

Pubic symphysis. The specimen consisting of the pubic symphysis and the two coxal bones, when not tested immediately, was surrounded with saline-moisten gauze and stored for no more than two hours. The pubic bones were placed into clamps which were contour shaped for the external surfaces of the bones (Fig. 41). The specimen was fixed against the clamp with a polyethylene tube-clad steel rod. The complex was then fastened into the materials testing machine.

Posterior cruciate ligament. The knee joint was kept in the hind limb until the testing. All structures except the posterior cruciate ligament were carefully removed from the knee joint. The posterior cruciate ligament was selected because its anatomical position permitted a more rapid dissection than the anterior one. The ligament is in a slightly oblique position, but as the specimens were of fairly uniform appearance the influence of skin on defor mation readings was assumed to be constant. The distant end of femur and proximal one of tibia were trimmed in length to fit the contour shaped brass clamps (Fig. 42). The clamps were firmly screwed together and then attached to the materials testing machine.

Muscle tendons. The tendons remained in situ until tested. From the lateral muscle group of the hind limbs a muscle tendon was carefully dissected

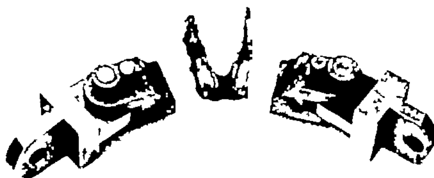


Fig 41 Contour shaped clamps for the pubic symphysis specimens. During the experiment the specimen is fixed towards the surfaces of the clamps with polyethylene tube clad steel bars.

out. The outermost of the peroneus muscle was cut away The peroneus digiti quinti muscle which arises from the proximal half of the shaft of the fibula and inserts upon the distal end of the fifth metatarsal bone was freed and its tendon taken for the experiment. The specimen was placed in two saw-toothed clamps with the distance between the edges of the clamps being 3 0 mm when attached to the testing machine. During the experiment the tendon was immersed in a buffered Ringer's solution, pH 7 4 at room temperature.

Skin. The dorsum of the rat was shaved with hair-clippers and a rectangular piece of the middorsal skin was removed. Strips of skin 2 mm wide were cut out using parallel mounted razors. The strips were at a right angle to the cranio-caudal axis. The specimen was then freed from the subcutaneous muscle and mounted in two saw-toothed clamps and mounted in the materials testing machine. During testing the specimen was immersed in buffered Ringer's solution pH. 7 4 at room temperature.

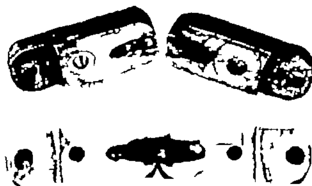


Fig. 42 Contour shaped clamps for the posterior cruciate ligament specimens. The upper parts of the clamps are in the background and the lower parts with a specimen are seen in the front

Results

Pubic symphysis

Young pregnant rats. The macroscopic appearance of the interpubic joint appears the same all through pregnancy. Microscopically (Fig. 43) there seems to be an increase in the ground substance, and the chondrocytes are somewhat more scattered and appear larger and rounded.

The specimens always failed at the junction between one of the bones and the cartilage plate, except for a few cases when one of the coxal bones was fastened too firmly in the clamp and accidentlally fractured. The specimen was discarded if there were any signs of bone fracture after the test.

The load-deformation relationships are presented in Figs. 44 and 45 and Table A VII. During gestation the deformation values for the start and end of the linear region increase. The maximum deformation, however, is only increased in the 21 day group compare to the non pregnant rats. The load



Fig 43 Photomicrographs of pubic symphyses of young rats (formic acid decalcified, htx stained) ( ) shows specimen from non-pregnant rat. (b) shows a specimen from one day after parturition. (11 x)

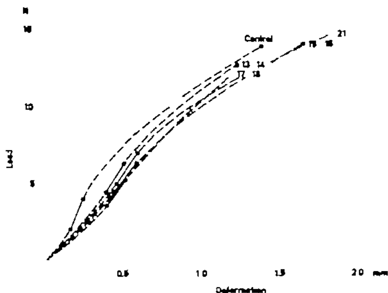


Fig. 44 Mean load-deformation curves of pubic symphysis specimens of young rats during gestation. The figures at the tops of the curves denote days of gestation.

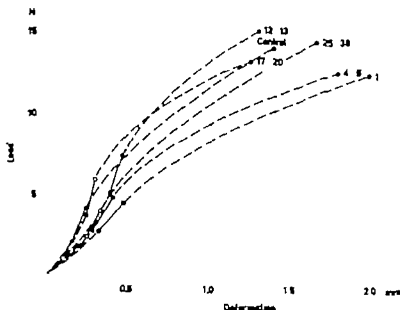


Fig 45 Mean load-deformation curves of pubic symphysis specimens of young rats during the post-partum phase. The figures at the tops of the curves denote day after parturition.

values for the start and end of the linear region is significantly increased in several gestational groups compared to the non-pregnant group. The maximum load is not influenced by gestation being in the same range in all groups as that of the controls. The "elastic stiffness" values are decreased for the 15 16 and 17 18 day groups and tend to decrease in the 19-20 day group compared to the non-pregnant rats. It is noticable that a few days before parturition the "elastic stiffness" values are greater than during the middle of gestation. The changes in the pubic symphysis during gestation result in larger deformation and lower load values in the first part of the load-deformation curves.

After parturition the deformation values for the start and end of the linear region vary without any clear trend. The values of the 2 3 day and the 12 13 day groups are significantly higher than those of the controls while the values of the other post partum groups are in the range of the non-pregnant animals. The deformation at maximum load remains unchanged also during the post partum period. After parturition the load values for the start and end of the

linear region vary almost in the same way as the deformation values for the same coordinates and the maximum load remains unchanged One day after parturition the "elastic stiffness" is significantly decreased compared to the non-pregnant group. This is also the case for the 7 9 day group but during the rest of the involution period, these values for elastic stiffness" are in the same range as the control group.

Old multiparous rats. As in the young animals the specimens failed at one of the bone cartilage junctions. Due to somewhat thinner and more fragile coxal bones in the old animals some of the specimens fractured in the clamps and thereby excluded from the series. Four bone fractures were noted in the non-pregnant group and three in the 4 pregnancies group. The biomechanical properties of the pubic symphysis are presented in Fig 46 and Table A VII If the pooled group of all multiparous animals is compared with the virginal group no differences are found for the deformation values. In the 5" pregnancies group the deformation value for the end of the linear region is signifi

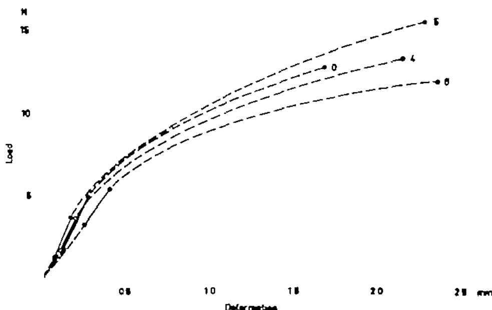


Fig. 46 Mean load-deformation curves of pubic symphysis specimens of old multiparous rats. The figures at the tops of the curves denote number of pregnancies.

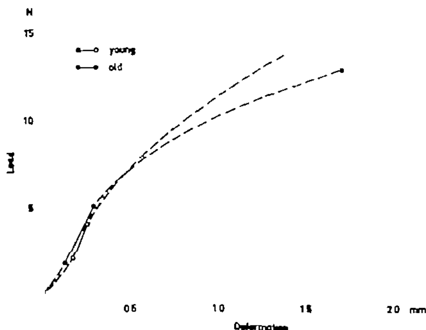


Fig 47 Mean load-deformation curves of pubic symphysis specimens of young and old virginal rats.

cantly lower compared to the virgins. The load value for the end of the linear region is decreased in the multiparous animals while the values for the start of the linear region, maximum load and "elastic stiffness" are not significantly different compared to the control group

Young and old virginal rats The influence of aging on the physical properties of the pubic symphysis (Fig 47 and Table A VII) is very slight. The values for all parameters are in the same range on the two groups except for the load value at the end of the linear region, which tends to increase in the old rats.

Posterior cruciate ligament

Young pregnant rats The specimens invariably failed in one of the bone ligament junctions. The maximum load value will thus express the condition of the bone-ligament junction more than the strength of the ligament itself

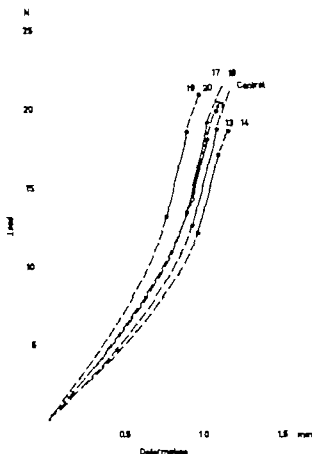


Fig 48 Mean load-deformation curves of posterior cruciate ligament specimens of young rats during gestation. The figures at the tops of the curves denote days of gestation.

The other parameters, the linear region and the "elastic stiffness" are more relevant for the properties of the collagenous framework in the ligament. The values are presented in Figs. 48 and 49 and Table A VIII

The deformation values during gestation are similar to the non pregnant rats except for two groups. In the 6-10 and 19-20 day groups lower values are recorded. The decreased deformation in the 6-10 day group is followed in the 13-14 day group by values in the range of the control group. During ges-

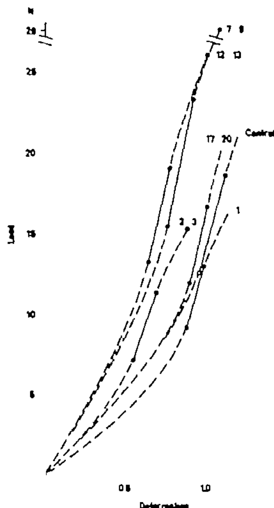


Fig 49 Mean load-deformation curves of posterior cruciate ligament specimens of young rats during the post-partum period. The figures at the tops of the curves denote days after parturition

tation the load values at the start and end of the linear region are in the range of the non pregnant animals. The maximum load values are also in the range of the controls except for the 13-14 day group and this value is significantly decreased The "elastic stiffness values are similar to that of the virginal group

During the first two weeks after parturition the deformation values for the start and end of the linear region are decreased in some of the groups. The value for the deformation at maximum load is decreased only in the 2 3 day group while the other groups are in the same range as the controls. The load values decrease significantly for the first three days after parturition followed by an increase in the 7 9 day group with values significantly higher than those for the non-pregnant animals. In the last post partum groups the load values are in the range of the control group. The elastic stiffness" is significantly decreased in the 2 3 day group, rises sharply in the 7 9 day group and again decreases to the value of the non pregnant animals in the 12 13 day group

Old multiparous rats The specimens invariably failed at one of the bone ligament junctions as in the young rats. The results from the biomechanical

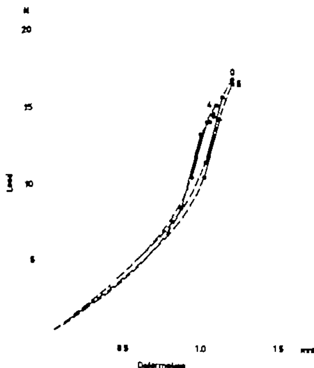


Fig. 50 Mean load-deformation curves of posterior cruciate ligament specimens of old multiparous rats. The figures t the tops of the curves denote umber of pregnancies.

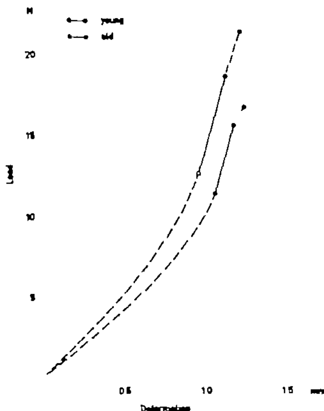


Fig. 51 Mean load-deformation curves of posterior cruciate ligament specimens of young and old virginal rats.

tests (Fig. 50 and Table A VIII) show no variation in the deformation values between the multiparous and virginal animals. Also the load and elastic stiffness values *for the multiparous animals are not significantly different* compared to the virginal rats.

Young and old virginal rats. The influence of aging on the biomechanical properties of the posterior cruciate ligament is shown in Fig. 51 and Table A VIII. The deformation values for the young animals are not different compared to those of the old group. The load values for the start and end of the linear region are not lower in the old virgins while the maximum load is significantly decreased in this group. The "elastic stiffness" tends to decrease in the old animals compared to the young ones. Thus the consequence of

Table XI Amount of collagen per millimeter specimen length of muscle tendons (mean values ± S.E.M.) from young and old rats.

	No of animals	mg/mm
YOUNG		
Control	10	0.0217 ± 0.0018
Gestational		
Days		
6–10	2	0.0170 ± 0.0005
13–14	6	0.0190 ± 0.0026
15–16	7	0.0262 ± 0.0023
17–18	7	0.0243 ± 0.0021
19–20	7	0.0297 ± 0.0021
21	3	0.0279 ± 0.0044
Post partum		
Day		
4–6	3	0.0301 ± 0.0063
13–15	4	0.0375 ± 0.0051
17–20	8	0.0361 ± 0.0042*
25–38	3	0.0374 ± 0.0015
OLD		
No of pregnancies		
0 = control	6	0.0281 ± 0.0060
4	14	0.0382 ± 0.0024
5	7	0.0360 ± 0.0024
6	3	0.0387 ± 0.0042
4 + 5 + 6	24	0.0381 ± 0.0015

*2P < 0.05 against control.

aging seems mainly to be reduced strength of the insertion of the ligament while the collagenous part is unaffected.

Muscle tendon

Young pregnant rats The specimens usually failed at the edge of one of the clamps because the pressure of the jaw edges weakens the tendon by thinning it out.

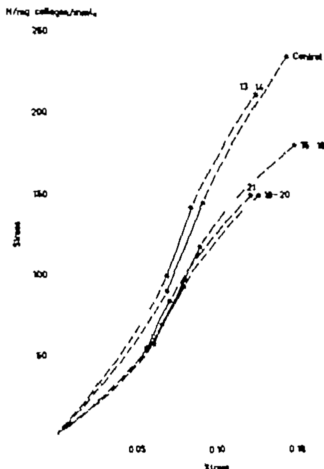


Fig 52. Mean stress-strain curves of muscle tendon specimens of young rats during gestation. The figures t the tops of the curves denote days of gestation.

Compared to the non-pregnant group the amount of collagen per unit specimen length (Table XI) decreases in the 6-10 day gestational group followed by significantly increased values in the 19-20 day group. During the post partum phase all the values are significantly increased except for the 4-6 day group. Thus no sign of a return to non pregnant values during the involution period studied can be found in these muscle tendons.

The stress-stain relationships for the muscle tendons during pregnancy are presented in Figs. 5? ad 53 and Table A-IX. Both during gestation and in the

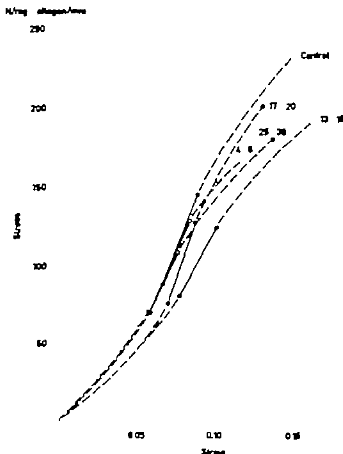


Fig. 53 Mean stress-strain curves of muscle tendon specimens of young rats during the post-partum period. The figures at the tops of the curves denote after parturition.

post partum period the strain values for the start and end of the linear region are in the same range as for non-pregnant rats. The maximum deformation varies somewhat more probably due to the clamping of the specimens. The stress values of the linear region during gestation compared to the non-pregnant group is increased in the 6-10 day group. In the later gestational groups these values are not different from non-pregnant groups except for the 19-20 day and 21 day groups in which the values for the start of the linear region are significantly decreased. The maximum stress values show the same pat

tern, although these values do not express the true ultimate tensile strength of the tendon. The "modulus of elasticity" in all gestational groups is not significantly different from the controls. During the post partum period the stress value for the start of the linear region in the 4-6 day group is significantly lower compared to the non pregnant animals. In the other post partum groups this value is in the same range as that of the controls. There are no differences in the values for the stress at the end of the linear region and the "modulus of elasticity" during involution compared to the non pregnant rats.

Old multiparous rats The specimens always failed at the edge of one of the

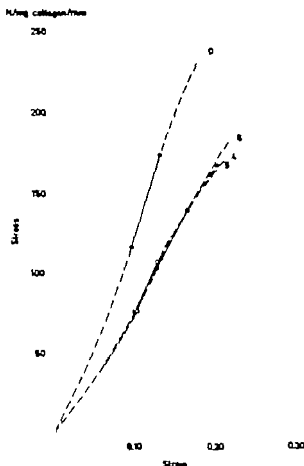


Fig 54 Mean stress-strain curves of muscle tendon specimens of old multiparous rats. The figures at the tops of the curves denote number of pregnancies.

clamps as did the tendons from the young animals. The amount of collagen per unit specimen length (Table XI) is not different between virginal and multiparous animals.

The values for the stress and strain at the start and end of the linear region and for the "modulus of elasticity" in the multiparous groups are not significantly different from the control group (Fig 54 and Table A IX)

Young and old virginal rats There is no different in the amount of collagen per unit specimen length between the two groups (Table XI) In the stress-strain relationship (Fig 55 and Table A IX) the strain value for the

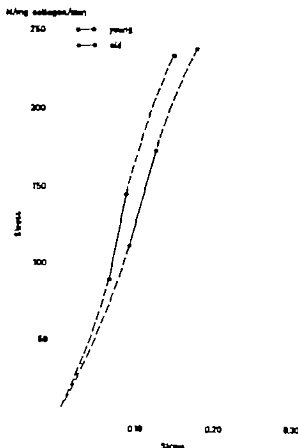


Fig 55 Mean stress-strain curves of muscle tendon specimens of young and old virginal rats.

Table XII Amount of ollagen per unit specimen length f skin specimens (mean values ± S.E.M.) from young and old ats.

	No of animals	mg/mm
YOUNG		
Control	10	0.2557 ± 0.0175
Gestational		
Days		
6–10	3	0.2266 ± 0.0199
13–14	7	0.2744 ± 0.0266
15–16	9	0.2916 ± 0.0183
17–18	6	0.2373 ± 0.0065
19–20	12	0.2541 ± 0.0170
21	4	0.2659 ± 0.0224
Post partum		
Days		
1–2	2	0.2745 ± 0.0672
4–6	3	0.3000 ± 0.0643
13–14	5	0.3473 ± 0.0416
16–20	8	0.2866 ± 0.0263
25–38	4	0.2431 ± 0.0237
OLD		
No of pregnancies		
0 = control	7	0.1299 ± 0.0144
4	13	0.1475 ± 0.0073
5	10	0.1815 ± 0.0135vv
6	4	0.1343 ± 0.0074
4+5+6	29	0.1570 ± 0.0067

$2P<0.10$ $2P<0.05$ against young control.
$2P<0.10$, vv $2P<0.05$ against old control

end of the linear region is significantly increased in the old group. The stress values show no differeces nor do the "modulus of elasticity"

Skin

Young pregnant rats. The amount of collagen per unit specimen length (Table XII) does not change during gestation. In the post partum phase the

mean values for the different groups are in the range of the controls, although the value for the 13-14 day group tends to increase.

The results of the mechanical tests on the skin during pregnancy are given as diagrams and stress-strain parameters in Figs. 56 and 57 and Table A X. During gestation the strain values for the start and end of the linear region and the maximum strain are not significantly different from the non-pregnant group. The stress value for the start of the linear region tends of increase in the 6-10 day group and is significantly increased in the 13-14 day group. Then there is a decrease in this value to the same range as the non pregnant group. The stress value at the end of the linear region is significantly increased in the 6-10 day and 13-14 day groups, but from the 15-16 day group this value is at the non-pregnant level although the value tends to increase in the 21 day group. The "modulus of elasticity" is significantly increased in the 6-10 day group but only tends to increase in the 13-14 day group. The value then decreases to the range of the control group until the 19-20 day when it again

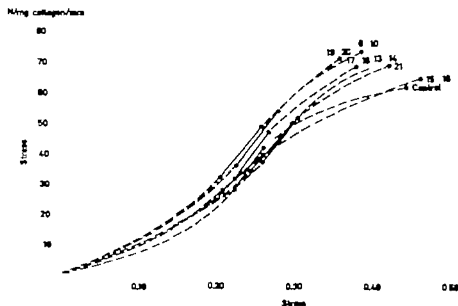


Fig. 56. Mean stress-strain curves of skin specimens of young rats during gestation. The figures at the tops of the curves denote days of gestation.

T ble XII Amount of ollagen per unit pe imen length f skin specimens (mean values ± S.E.M) from yo g and old rats

	No of animals	mg/mm
YOUNG		
Control	10	0.2557 ± 0.0175
Gestational		
Days		
6–10	3	0.2266 ± 0.0199
13–14	7	0.2744 ± 0.0266
15–16	9	0.2916 ± 0 0183
17–18	6	0.2373 ± 0.0065
19–20	12	0.2541 ± 0.0170
21	4	0.2659 ± 0.0224
Post partum		
Days		
1–2	2	0.2745 ± 0.0672
4–6	3	0.3000 ± 0.0645
13–14	5	0.3473 ± 0.0416
16–20	8	0.2866 ± 0.0263
25–38	4	0.2431 ± 0.0237
OLD		
No of pregnancies		
0 = control	7	0.1299 ± 0.0144**
4	15	0.1475 ± 0.0073
5	10	0 1815 ± 0.0135▾▾
6	4	0.1343 ± 0.0074
4+5+6	29	0.1370 ± 0.0067

2P<0 10 2P<0.05 against young control.
▾ 2P<0.10 ▾▾ 2P<0.05 against old control.

end of the linear region is significantly increased in the old group. The stress values show no differeces nor do the "modulus of elasticity"

Skin

Young pregnant rats The amount of collagen per unit specimen length (Table XII) does not change during gestation. In the post partum phase the

parturition. As reported in Part III the wet weight per unit length of rat tail tendons does not change during pregnancy and thus it might be assumed that there are different patterns of reactivity in the two types of tendons.

During gestation no changes are found in the strain values. The stress values are increased in the 6-10 day group and decreased but only at the start of the linear region in the last two gestational groups compared to the controls. During the post partum period all parameters are in the same range as the non-pregnant animals except for a decrease in the 4-6 day group. For the posterior cruciate ligament a decrease in load values is found during the early post partum period, while for the muscle tendon there is a decrease during late gestation. One explanation for this phenomenon might be an increase in ground substance or its water binding capacity in the tendon and the ligament. The collagen fibers are then pushed apart resulting in lower load and stress values and an increase in the visco-elastic properties. Bryant et al (1968) could not find any increase in the per cent of hexosamine in freeze-dried rat tail tendons studied almost daily in the period from six days before to eight days after parturition. However analysis of other ground substance components is not reported.

In multiparous animals the strain, stress and modulus of elasticity" values are not different compared to the virginal animals. The amount of collagen per unit length is not affected by repeated pregnancies either In old virginal animals the strain value at the linear region is increased compared to young ones, but all other biomechanical parameters as well as the amount of collagen per unit length are the same between the groups. The physical properties of the muscle tendon and also the collagenous part of the knee joint ligament are generally not changed due to increased age for the time period studied.

Skin

Young p egnant rats The amount of collagen per unit specimen length does not differ during pregnancy compared to the non-pregnant animals. This is some what contrary to Müller (1951) who found from histologic studies that during gestation the dermis in rat becomes thick and the collagen fibers are closely packed Bryant et al. (1968) found an increased percentage of hydroxyproline in freeze-dried skin of rat studied in the period from 6 days before to 8 days after parturition.

The strain values are unchanged but the stress values are increased during the first half of gestation following which they return to the same range as the controls. These changes partly parallel those of the muscle tendons in which increased stress and unchanged strain values are found at the same period of gestation. In skin increased "modulus of elasticity" is also found close to par turition. Hormonal treatment of intact mature female rats has shown that both estradiol and progesterone increase the maximum load of skin as well as of tail tendons (Vogel, 1970) It is thus probable that these changes in the physical properties of collagenous tissues during pregnancy are mediated by sex hormones. It is also reasonable to assume that biomechanical parameters other than the above-mentioned can be influenced by these hormones. Preg nancy is a very complicated physiological process with intricate interactions, and deductive conclusions from model experiments where only one or a few factors have been analyzed are thus uncertain.

Old multiparous rats The amount of collagen per unit specimen length is the same in multiparous as in virginal rats. During a single pregnancy there is no change in this parameter and this agrees well with the finding that there are no detectable cumulative changes.

The biomechanical properties of the collagenous framework are somewhat changed by repeated pregnancies manifested in increased strain values. The other parameters, however show no differences between the multiparous and virginal rats. This results in decreased stiffness in the skin of the multiparous animals.

Young and old virginal rats Mendoza and Milch (1964/65) Fry et al. (1964) and Vogel (1973) reported that the ultimate tensile strength increased with age This is also confirmed in the present investigation. The strain values are unchanged but the stress and modulus of elasticity" values are signifi cantly increased in old virginal animals.

The collagenous framework of the skin becomes stronger during aging, and this process is only slightly modified by repeated pregnancies. The amount of collagen per unit specimen length decreases about 50 per cent in the old animals. During aging the collagen concentration in rat skin increases con tinuously as measured in per cent of wet weight (McGavack and Kao 1960) This is contrary to Vogel (1973) who found maximum concentration of hydroxyproline per gram fresh weight at the age og 4 months, followed by a slight decrease during aging Fry et al. (1964) found a 40 per cent decrease

in the skin collagen concentration on wet weight basis, when comparing 10 and 20 months old rats. There is also slight reduction in the wet weight concentration of other proteins from about 8 months of age onward (McGavack and Kao 1960) The skin in old rats is thus somewhat atrophic but in spite of this its force resisting properties are more pronounced than in young rats.

Part III

THERMAL REACTIVITY IN RAT TAIL TENDONS

Introduction

It is an old observation that collagen melts and its fibrous structure shrinks to a rubbery one when exposed to heat. A collagen fiber heated to above 58 C shrinks to about one forth of its original length (Verzár 1964) The thermal shrinkage is a phase transition involving melting of crystalline regions (Flory and Garret, 1958) Physical means other than heat can also be used for studying the collagen fiber contraction. Banga et al. (1956) induced this phenomenon by immersing the tendon fiber in a 40 per cent solution of potassium iodide, while Elden and Webb (1961) used 8 molar urea. Other concentrated solutions of electrolytes have also been used. Standardized measurements of the physical events in this transformation of the collagenous structure can be performed in two ways (1) isotonically against a small counterweight that straightens the specimen but does not prevent shrinkage and (2) isometrically measuring the tension that develops. It was first shown by Verzár (1955) that the degree of thermal shrinkage was dependent on age tendon fibers from older rats tail tendons shrank more than those from younger ones and there was a fairly good correlation to increasing age. It has also been shown (Verzár 1964) that the tension developed in isometric thermal contraction increases with age. There are a great number of investigations in which the above mentioned methods have been used to study the biological" age and aging of collagen fibers under varying experimental conditions, such as undernutrition (Chvapil and Hruza, 1959) ionizing radiation (Verzár 1964) and pregnancy (Árvay and Takács, 1964/65)

Young pregnant rats. Árvay and Takács (1964/65) measured the isotonic thermal contraction in rat tail tendons from animals divided in six age groups ranging between 4 and 13 months. Gestational animals from these groups were investigated and one of the groups was also studied on the 3-4 day after

parturition. On the 17 20 day of gestation increased tension was found in groups with animals aged 4 5 6 and 10 months. In the last group increased tension was found also on the 7 10 day of gestation. After parturition there was a reduction in the tension, but the mean value was still increased in comparison to the controls. They concluded that pregnancy enhanced the biological aging" of collagen although this effect was reversible to a certain extent. Viidik et al. (1972) investigated tail tendons in five to six months old rats during the first 15 days of the post partum period with thermal isometric contraction test. The tension was increased compared to the non pregnant group and no tendency for a decrease during late post partum was found.

A few other studies to evaluate the distant effects of pregnancy on the physical properties of the collagenous framework have been made on the pubic symphysis (Storey 1957 Crelin and Brightman, 1957) dermis (Muller 1951) and bone (Currey and Hughes, 1973 Currey 1973) which was discussed in part II.

Old multiparous rats With increasing age the properties of collagen undergo changes resulting in increased resistance to physical procedures such as thermal denaturation, swelling in acid, solubility and digestion by enzymes. This has been attributed to an accumulation of covalent intermolecular cross-links (Verzár 1964 1969 Elden, 1964) In the rat tail tendon increased cross-linking with age has been demonstrated by Heikkinen and Kulonen (1964) and Steel and Everitt (1970)

Árvay et al. (1963) investigated thermal contraction of the tail tendon from 24 months old rats which had had about eight pregnancies. They found in the multiparous rats both the thermal contraction of tail tendons to be more pronounced and a lower amount of extractable collagen in comparison to virginal ones. The multiparous rats had an increased mortality at the age of 24 months, as 50 per cent of the original number of these animals were dead compared to 30 per cent in the virginal control group of the same age.

While it is well established that a pregnancy and the following involution period cause profound morphological changes in the prime target area, i.e. the genital tract (Cf Part I) the influence of this phenomenon on distant collagen is less evident *(Cf Part II)* Analysis of the time sequence of changes in a pregnancy cycle and the cumulative effects of such cycles, as reflected in the thermal reactivity of tail tendon fiber may give more information on

the behavior of distant collagen than biomechanical measurements because of its greater precision.

Materials and methods

Experimental procedure The tail was cut off at its base and in addition a small segment was removed from the distal end. Collagen fiber bundles were carefully pulled out in the distal direction, the skin remaining intact. The fibers were first weighed in sealed tubes to avoid evaporation and thereafter the lengths were measured after the fibers were straightened out but not stretched. The length of the fibers was about 7-8 cm and the weight 4-5 mg The specimens were stored up to one hour in a tris buffered Ringer's solution, pH 7 4 at room temperature until the experiment.

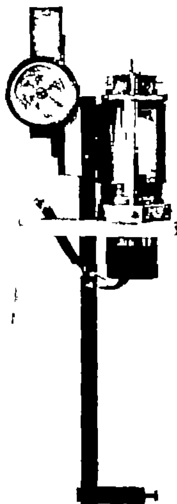
The isometric contraction relaxation tests were performed in an apparatus described by Viidik (1968b) In this (Fig 60) the tendon is mounted vertically between two clamps, the upper one suspended from a stainless steel blade via a rod on which the core of an inductive differential transformer is fastened (Bofors RRL-1 ±3 mm) The lower clamp is fastened to an adjustable arm. With an appropriately stiff steel blade a displacement of the core by 0.2 mm or less per 10^{-4} N tension gives a satisfactory reading as a mV d.c. signal on a digital voltmeter (Oltronix DPM 319) and ensures that the shortening is negligible in the 7 9 cm long fibers in these isometric experiments.

The specimen was mounted in the clamps and immersed into a buffered Ringer's solution, pH 7 4 at room temperature while an initial tension of 2×10^{-3} N was set. The clamping system was then immersed in the same type of Ringer's solution that was preheated to 62 C in a vessel placed in an outer waterbath and the temperature maintained constant. The tension was read versus time in tenths of minutes. Calibration was done before and after the experiments and constant results were obtained The tension values were normated to fiber fresh weight per unit fiber length.

Results

Young pregnant rats The weight per unit length (mg/cm) of the tendon during the pregnancy is almost the same in all groups showing no significant differences (Table XIII)

Fig 60 Apparatus for measuring isometric thermal contraction in rat tail tendons. For explanation see text.



A typical contraction-relaxation curve for the mean of five tail tendons from a non pregnant rat is shown in Fig 61 The rapid contraction which

Table XIII Weight per unit length (mean value ± S.E.M.) of tail tendons in young and old rats.

	No of animals	mg/cm
YOUNG		
Control	15	0.615 ± 0.028
Gestational Days		
6–10	5	0.595 ± 0.079
13–14	9	0.584 ± 0.025
15–16	9	0.563 ± 0.029
17–18	7	0.611 ± 0.037
19–20	13	0.579 ± 0.030
21	4	0.601 ± 0.047
Post partum Day		
1–2	3	0.575 ± 0.057
3–4	4	0.617 ± 0.037
5–7	3	0.602 ± 0.034
12–15	9	0.584 ± 0.042
16–20	9	0.645 ± 0.052
25–38	4	0.639 ± 0.088
OLD		
No of pregnancies		
0=control	9	0.739 ± 0.044
4	15	0.678 ± 0.036
5	10	0.628 ± 0.037v
6	4	0.583 ± 0.055vv
4+5+6	29	0.651 ± 0.024vv

** 2P<0.05 against young control.
v 2P<0.10 vv 2P<0.05 against old control.

ensues first is followed by a relaxation phase during a longer time interval. The tension at maximum contraction of the control animals was lower than that of the gestational rats Figs. 62 and 63 and Table A XI. During early gestation there is a significant increase in the isometric contraction tension followed by a decrease in the 13-14 day group to the same level as the controls. The values remain in this range until the 21st day when an increase is noted



Fig 61 Contraction-relaxation curve for the mean of five tail tendons of non-pregnant young rat. The tension values are normalized to milligrams fresh weight per cm tendon length and plotted against time.

again. The time to reach the maximum tension in the 6-10 day group is increased compared to the non pregnant group. After a decrease in the 13-14 day group to the range of the controls the value increases again in the 17 18 day and 19-20 day groups. In the 21 day group, however no increase is noted. One day after parturition the tension is not significantly different from the control group but increased tension values are found in the 3-4 day post partum group and in the rest of the post partum groups studied. Thus during the latter half of the involutional period no reversibility towards the non-pregnant values is seen. After parturition the time to each the maximum tension is increased in the 3-4 day group but this is not seen in the 5-7 day group. In the last three post partum groups this parameter is increased and no return to the non-pregnant level is seen here either

Old multiparous rats. The weight of the tendon per unit length for the pooled multiparous rats compared to the control group (Table XIII) tends

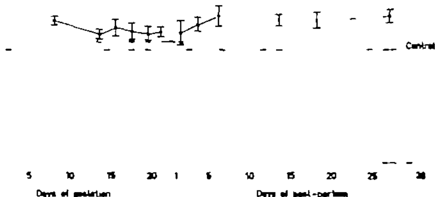


Fig. 62 Maximum isometric thermal contraction values for tail tendons of young rats d ring pregnancy The tension values are normalized to milligram fresh weight per cm tendon length. (Mean values ± S.E.M)



F g 63 Time to reach the maximum isometric thermal contraction values for tail tendons of young rats during pregnancy (Mean values ± S.E.M.)

to decrease and the value for the "6" pregnancies group is significantly lower

The maximum tension and the time to reach this value is presented in Figs. 64 and 65 and Table A XI for the different groups. The maximum ten-

The time to reach maximum contraction is significantly increased for all

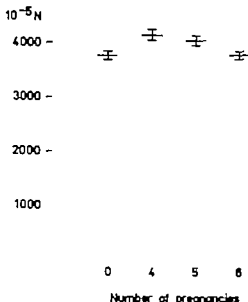


Fig. 64 Maximum isometric thermal contraction values for tail tendons of old multiparous rats. The tension values are normalised to milligram fresh weigth per cm tendon length. (Mean values ± S.E.M.)

sion in the "4" pregnancies group is significantly increased, while the tension in the "5" pregnancies group only tends to increase. The value for the pooled multiparous rats is also significantly higher. The tension in the group with six previous pregnancies is almost the same as that of the controls.
the multiparous groups as compared to the control group. The greatest value is found in the "6" pregnancies group.

Young and old virginal rats. In the old animals compared to the young ones the maximum contraction is almost three times greater (Figs. 61 and 63 and Table A XI) and the time to reach this value about four times longer (Figs. 62 and 64 and Table A XI)

Discussion

Young pregnant rats. The extragenital connective tissue does not fullfill any known physiological function connected with pregnancy. These collagenous

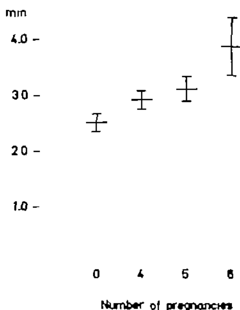


Fig 65 Time to reach the maximum isometric contraction values f tail tendons of old multiparous rats. (Mean val es ± S.E.M.)

structures are not target tissues during gestation and the changes noted are less pronounced than in the genital tract. As discussed in connection with the influence of pregnancy on the biomechanical properties of skin, the changes that take place might be mediated via sex hormones, primarily estrogen and progesterone. Other hormones such as relaxin, corticosteroids and thyroxin might also be of importance The relationship between estrogen and progesterone varies during pregnancy The ovarian secretion of estrogen (Yoshinaga et al. 1969) and of progesterone (Hashimoto et al. 1968) have been studied in pregnant rats. The level of estrogen is relatively stable during gestation until a few days before parturition. It increases about 3-4 times and then rapidly decreases after parturition to almost a zero level. Progesterone is secreted in increasing amounts until the 14th day of gestation when a peak value is reached. During the late term progesterone secretion is decreased and reaches almost zero level just before parturition. The post partum period is dominated by progesterone secretion.

There might be a connection between the increased tension during early gestation and the post partum phase and the increased amount of progesterone. The slightly lower tension values from the 13th day of gestation to parturition might be explained by interaction of estrogen and progesterone. According to Vogel (1970) progesterone given to intact rats has a biphasic effect on the maximum tensile strength of rat tail tendons. Decreased values were noted during the first days of treatment followed by increased values. This was found both with high and low doses. Estrogen also resulted in increased tensile strength but without the decrease in the beginning (Vogel, 1970) Árvay and Takács (1965) administered estrogen and progesterone to intact rats for 16-17 days but could find no effect on tail tendons as tested by the thermal isotonic test. The knowledge of the action of sex hormones on extragenital connective tissue is insufficient to explain the changes found during pregnancy

Old multiparous rats. Árvay et al. (1963) reported that tail tendons from old multiparous rats showed an enhance isotonic contraction compared to virgins of the same age. However their animals came from a random pool of rats with an unknown number of previous pregnancies for each individual although the mean number of pregnancies for the pool was known. Only a few results from the older population were reported In the present investigation the number of pregnancies for each rat is known and recorded.

The maximum tension for collagen fibers in isometric thermal reaction as well as the time to reach this point increase with age (e.g Verzár 1963 1964 Viidik, 1969) This is also confirmed in the present investigation. The increased maximum contraction and time to reach this value is due to the enhanced crystalline structure of the collagen molecules, which requires a greater amount of energy and longer time to break the intra and intermolecular bonds. Florey (1956) showed that an increased number of cross-links increases the denaturing temperature. Viidik (1969) demonstrated that a higher temperature breaks the cross-links more effectively than a lower temperature and a higher tension develops with identical collagen fiber specimens. The difference in maximum tension between the 4 pregnancies group and 5" and 6 pregnancies groups might be explained by the fact that a higher temperature than 62 C is required for the complete denaturation of the specimen for the latter groups. The increased time required to reach maximum contraction supports this explanation.

The results show that pregnancy and the cumulative effect of repeated

tative but also qualitative changes had occured in the collagenous framework of the cervix. In the vaginal wall specimens the size increased and amount of collagen per unit length decreased. The length but not deformation values were increased and the load values were reduced about three times during gestation. The strain values were not generally reduced. The stress values at the time of parturition were decreased to half the non-pregnant values, but the "modulus of elasticity" was not changed. Thus also in the vaginal wall changes in the force resisting properties of the collagenous framework were found.

In the genital tract during gestation quantitative and qualitative changes of the collagenous framework occured that for the uterine horns could be called an adaptive process to the growing fetuses and for the uterine cervix and vagina an anticipation to the passage of the fetuses.

During the post partum period in the uterine horn specimens increased deformation and load values were recorded for the first two days after parturition followed by a decreased during the period, which biochemically is characterized by collagen catabolism. Later the values were restored towards the level of the non pregnant rats. The strain and stress values changed in the same way indicating derangements of the collagenous framework and loss of force resisting properties when the collagen was broken down. In the uterine cervix the deformation and load values also increased soon after parturition followed by a decrease but the load values at this time never reached the low level recorded just prior to parturition. The stress-strain relationship changed in the same way. A restoration towards the range of the non-pregnant animals was noted in the last post partum groups. The deformation values decreased somewhat but the load values of the vaginal wall specimens increased slightly after parturition and these values were not followed by the decrease noted for the other parts of the genital tract. The stress and strain values showed the same pattern as the load values reaching the non pregnant level in the last post partum groups. The collagenous framework of the vaginal wall was not subjected to as pronounced derangements as other parts of the genital tract during the involution period.

During gestation the physical properties of the pubic symphysis were changed. In the load-deformation curves a more pronounced toe-part appeared, the start of the linear region ensued later and the "elastic stiffness" was decreased compared to non-pregnant animals. During the first two weeks of the post partum period the properties of the pubic symphysis with some ex

ceptions remained unchanged compared to those of gestation. Then the values approached the range of the non-pregnant rats. The biomechanical properties of the posterior cruciate ligament were generally not affected during gestation but the load values were reduced for the first three days after parturition. They then increased significantly above the level of the non pregnant animals after the first week of the post partum period. In the last post-partum groups the values had returned to the range of those of the control group. Muscle tendons showed reduced stress values at the end of gestation. Also during the first week after parturition the stress values were reduced but during the rest of the involution period studied no changes were found compared to non-pregnant rats. In the skin specimens the stress values increased during the first half of gestation but then the values returned to the range of the non pregnant rats. Increased stress values were found in the first and last of the post partum groups studied. In the rest of the groups no changes were found.

Being associated with the process of parturition the biomechanical changes of the pubic symphysis might be considered as an anticipation to this process. After parturition the organ underwent a restoration to non-pregnant conditions. For the parallel fibered collagenous tissues changes were noted mainly around the time of parturition as decreased force resisting properties. In skin on the other hand a slight increase was noted at the same time. These changes might be a distant response to the profound changes of the collagenous framework of the genital tract but are without physiological significance.

Biomechanical investigations are limited in the sense that only fairly large changes occuring on the molecular level can be recorded. In the organs discussed above in addition to collagen there are other tissue components that might influence or modify the physical properties. The tail tendons of the rat consist of almost pure collagen and an estimation of the entropy of the fibers is possible by measuring its thermal reactivity. This method has a greater precision than tensile strength measurements and will allow safer conclusions of the behavior of the distant collagen during pregnancy. Analysis of the thermal isometric contraction of tail tendons from the same rats showed that the maximum contraction was increased or tended to increase in all except one group during gestation. The time to reach the maximum contraction value was increased during early and late gestation. During the post partum period the maximum contraction was increased in all groups except for the group

just after parturition. The time to reach the maximum contraction value was also not increased in the first group after parturition but increased in the rest of the groups during the post partum period. The entropy of the collagen in the tail tendons was generally increased during pregnancy. This supports the previously discussed findings that a distant collagen effect seems to occur.

As the restoration during the post partum period in some instances was incomplete the influence of repeated pregnancies on the physical properties of the connective tissue was also studied. The old multiparous rats had had 4, 5 or 6 pregnancies, and were 22-23 months old when tested. They were matched against a virginal group of the same age.

The uterine horn specimens in the multiparous rats had decreased load values but the stress values were unchanged compared to the virgins. The strain values were reduced in the multiparous animals indicating a stiffer framework. A comparison between young and old virginal rats showed that in the old animals the load values were increased but the stress values were decreased. In the old multiparous rats but not in old virginal ones modulus of elasticity was decreased compared to young virgins. Thus aging qualitatively changes the collagenous framework and multiparity could to a certain degree influence this process.

The uterine cervix specimens of the multiparous animals had the same load and stress values as the old virgins. The strain values showed a tendency to increase in the multiparous rats. In old virginal rats compared to young ones the length values were increased, the load unchanged and the stress values decreased. In the old multiparous rats but not in the old virgins the "modulus of elasticity" was decreased compared to the young virginal rats, indicating the same pattern of aging as in the uterine horns.

Vaginal wall specimens of multiparous rats showed decreased maximum load and maximum stress values. These parameters were not different between young and old virginal rats. Repeated pregnancies but not aging influenced the force resisting properties of the collagenous framework here.

The influence of repeated pregnancies on the physical properties of extragenital collagenous structures was also investigated. In the pubic symphysis of multiparous rats the load values for the end of the linear region were decreased compared to virginal rats. No other biomechanical parameters were different. No differences were found between young and old virginal rats. In the posterior cruciate ligaments no differences were found between multipa

rous and virginal rats. In old virginal rats the maximum load was decreased and the "elastic stiffness" tended to decrease compared to young virgins. As the ligament failed at one of its bone attachments the change in maximum load indicated changes in the bone-ligament junction. Age changes in the collagenous part of the ligament were small. Between young virginal and old multiparous rats differences were found with lower values for the start and end of the linear region and decreased "elastic stiffness" were noted in the old multiparous animals compared to the young virginal rats. Thus repeated pregnancies more than age seemed to change the physical properties for this ligament. In muscle tendons no changes were found between old multiparous and virginal rats. In old virginal rats compared to young ones the strain value for the end of the linear region was increased but the other parameters were not different. The strain values were increased and "modulus of elasticity" decreased for the multiparous rats compared to young virgins. Thus repeated pregnancies also influenced the physical properties of muscle tendons more than aging

In skin specimens from old multiparous rats the strain values were increased compared to old virginal rats. The stress values were not different. In old virginal rats the stress values and "modulus of elasticity" were increased compared to young virgins. These parameters were also increased for old multiparous rats compared to the young virginal ones. Age changes in the skin of the rat were more pronounced than in other extragenital structures and it was thus difficult to assess the influence of repeated pregnancies.

In the old multiparous rats the maximum thermal contraction of tail tendons was increased compared to the virgins. This was also the case for the time to reach this value. In old virginal rats compared to young ones the maximum contraction and the time was increased very noticeably Thus repeated pregnancies enchance the "biological aging" of the collagenous framework in the tail tendons.

It can be concluded that changes in the physical properties of the collagenous framework in the reproductive tract are part of an adaptive physiological process and the changes found can be interpreted in terms of this. On the other hand changes in the biomechanical properties of the extragenital connective tissue except for the pubic symphysis are more difficult to explain from a functional point of view They seem to be a side effect distant to the functionally meaningful changes in the reproductive tract.

Repeated pregnancies resulted in changed biomechanical properties of both genital and extragenital collagenous structures. In several of the structures tested the results indicated enhanced "biological aging"

ACKNOWLEDGEMENTS

The present study was carried out at the Department of Anatomy University of Göteborg and at the Department of Connective Tissue Biology Institute of Anatomy University of Aarhus.

Especially I wish to express my thanks to my chief Professor A. Viidik, M D for his support, advice and guidance in all aspects of my work.

I am grateful to tekn. lic. L. Sonnerup Chalmers Institut of Technology Göteborg for advice on technological aspects of this work.

My thanks are also due to Professor H. Neustein, M. D for valuable criticism.

Likewise I want to thank Mrs. Ulla-Brit Carlsson and Miss Anne Li Johansson for their skilfull technical assistance.

I am much indebted to Mrs. Inge Marie Ketley for excellent secreterial work.

I also want to extend my sincere thanks to A. Meier medical photographer and Mrs. K. Sorensen medical illustrator

The experimental investigation was supported by grants from the Medical Faculty of the University of Göteborg Göteborgs Läkaresällskap and the Danish Medical Research Council (No 512 – 1993/72) to which I am greatly indebted.

REFERENCES

ADAMS, A., Effect of excercise upon ligament strength. *Res Quart. Amer Ass Hlth phys Educ* 1966. *37* 163–167

ALBERT E. N The effect of pregnancy of the elastin of uterine arteries. University Microfilms, Ann Arbor 1966.

ÁRVAY A. and J TAKÁCS, Änderung der Thermokontraktion von Kollagenfasern während der Gravidität. Die Wirkungen der Gravidität auf die Geschwindigkeit des biologischer Alterns von Kollagen. *Gerontologia.* 1964/65 *10* 146–156.

ÁRVAY A. and J TAKÁCS, The influence of hormone changes during pregnancy on the velocity of collagen aging. *Gerontologia.* 1965. *11* 188–197

ÁRVAY A., J TAKÁCS and F VERZÁR, Der Einfluss von Graviditeten auf das Altern des Kollagen (Versuche an Ratten) *Gerontologia.* 1963 *7* 77–84

BALOGA, I J BALÓ and D SZABO Submicroscopic structure of collagen fibres. Their contraction and relaxation. *Acta mo ph. Acad. Sci. hu g* 1956. *6* 391–402

BEAR, R. S X ray diffraction studies on protein fibers. I The large fiber-axis period of collagen. *J Amer Chem. Soc* 1944 *66* 1297–1305

BERGMAN I and R. LOXLEY Two improved and simplified methods for the spectrophotometric determination of hydroxyproline. *Anal. Chem 1963 35 1961–1965*

BIGGERS, J D C. A. FINN and A. MCLAREN, Long term reproductive performance of female mice. I. Effect of removing one ovary *J Reprod Fertil.* 1962. *3* 303–312

BRANDES, E. and E. ANTON An electron microscopic cytochemical study of macrophages during uterine involution. *J C ll. Biol.* 1969 *42* 450–461

BROWN J A., Structural and mechanical studies on human skin. Ph. D Thesis, University of Strathclyde 1971 Cited by P E. MILLINGTON, T GIBSON, J H. EVANS, and J C. BARBENEL 1971

BRYANT W M., J E. GREENWELL and P M. WEEKS, Alterations in collagen organization during dilatation of the cervix uteri. *S g Gyn c Obstet* 1968. *126* 27–39

BUCHER, O Histologie und mikroskopische Anatomi des Menchen. 3rd. ed. Huber Bern. 1962

BURACK, E., J M. WOLFE W LANSING and A. W WRIGHT The effect of age upon the connective tissue of the uterus, cervix and vagina of the rat. *Cancer Res* 1941 *1* 227–235.

CHRISMAN, O D The ground substance of connective tissue. A review of some recent advances in normal and pathologic physiology of the mucopolysaccharides. *Clin. Orthop.* 1964 *36* 184–193

CHVAPIL, M. and Z. HRUZA, The influence of aging and undern trition on chemical contractility and relaxation of collagen fibers in rats. *Gerontologia.* 1959 *3* 241–252.

CRELIN E. A. and M. W BRIGHTMAN, The pelvis of the rat. its response to estrogen and relaxin. *Anat. Rec.* 1957 *128* 467–483

CULLEN B. M. and R. D HARKNESS, Stimulation by distention of collagen formation in the rat's uterus. *J Physiol.* (Lond.) 1959 *148* 6P

CURREY J D Interaction between age, pregnancy and lactation, and some mechanical properties of the femora of rats. *Calcif Tiss. Res* 1973 *13* 99–112.

CURREY J D and S. M. HUGHES, The effects of pregnancy and lactation on some mechanical properties of the femora of the rat. *Calcif Tiss Res* 1973 *11* 112–123

CURTIS D H., The effect of chemical cross-linking agents on the mechanical properties of rat tail tendon. University Microfilms Inc., Ann Arbor 1963

DATTA, I. G., J N KARKUN and A. B. KAR, Studies on physiology and biochemistry of the cervix Changes in the cervix of rats during estrus cycle. *Acta biol. med germ.* *1968. 20 147–154*

DE VAAL, O M. Extensibility of the ostium uteri in th rat. *Acta b ew neerl. Physiol.* 1946. *14* 79–81

EISEN, A. Z., E. A. BAUER and J J JEFFREY Animal and human collagenases. *J invest. Derm.* 1970. *55* 359–373

ELDEN H. R., Aging of rat tail tendons. *J Geront* 1964 *19* 173–178

ELDEN, H. R., Cohesion of collagen fibers III. Modulus of elasticity and tensile-strength of aging rat tail tendon. 1969 Personal communication to Viidik.

ELDEN H. R. and R. J BOUCEK, Investigations on the aging process by physical-chemical means-summary In. N W SHOCK (ed.): *Biological aspect of ging* Columbia University Press. New York. 1962. pp. 334–342.

ELDEN H. R. and G WEBB, Extendability of tendons undergoing contraction relaxation. Nature (Lond.) 1961 *192* 742–744.

FADEETA T The helical collagen fibres in the uterus IV Extracellular studies of the uterus. *Amer J Obstet Gynec* 1964 *89* 1026–1039

FINLAY B., Scanning electron microscopy of the human dermis under uniaxial strain. *Biol. med Eng g* 1969 *4* 322–327

FIORI C. A., S. M. FITCH and R. D HARKNESS, Collagen content of barren and previously pregnant uterine horns in old mice. *J Reprod. Fertil.* 1963 *6* 405–407

FITTON JACKSON S., The morphogenesis of collagen. In B. S. GOULD (ed.): *T eature ollagen. Vol. 2 Biology of collagen part B* Academic Press. London. 1968. pp. 1–66.

FLORY P J Theory of elastic mechanisms in fibrous proteins. *J Amer chem. Soc* 1956 *80* 4836–4848.

FLOREY P J and R. R. GARRETT Phase transitions in collagen and gelatin systems. *J Amer chem Soc* 1958. *78* 5222–5234

FORRESTER, J C., B. H ZEDERFELDT T L. HAUES and T K. HUNT Mechanical, biochemical and architectural factors in repair In J E. DUNPHY and W VAN WINKLE (eds) Repair and Regeneration. McGraw-Hill. New York. 1969 pp 71–87

FRIEDEN E. H. and F L. HISAW The biochemistry of relaxin *R c at P gr Hormone Res.* 1953 *8* 333–378.

FRISÉN, M., M. MAGI, L. SONNERUP and A. VIIDIK, Rheological analysis of soft collagenous tissue. Part I: Theoretical considerations. *J Biochem.* 1969a. *2* 13–20

FRISÉN M. M. MÄGI, L. SONNERUP and A. VIIDIK, Rheological analysis of soft collagenous tissue. Part II Experimental evaluations and verifications. *J Biomech.* 1969b *2* 21–28.

FRY P M L, R. HARKNESS and R. D HARKNESS, Mechanical properties of the collagenous framwork of skin in rats of different ages. *Amer J Physiol.* 1964 *206* 1425–1429

GALANTE, J P Tensile properties of the human lumbar annulus fibrous. *Acta orth p. Sca d Suppl.* 1967 *100* 7–91

GAARDELL, S., L.-Å. FRANSSON and D HEINEGÅRD Chemical structure of extracell lar glycosaminoglycans (Mucopolysaccharides) and proteoglycans. In A. ENGEL and T LARSSON (eds.) *Thule International Symposia Aging of C nnectiv and Skeletal Tissu s.* Nordiska Bokhandeln. Stockholm. 1969 pp. 49–64

GIBSON, T Biomechanics in plastic surgery In. R. M. KENEDI (ed.) *Biomechanics and Related Bio-Engin ering Topics* Pergamon Press. Oxford. 1965 pp. 129–134

GRANT R. A., Chemical changes in the terus of the rat during late pregnancy and post-partum involution. The effects of lactation and hormono treatment. *J R prod Fertil.* 1965 *9* 285–299

GOULD B. S Collagen biosynthesis. In B. S GOULD (ed.) *T eatise on ollagen. Vol. 2 Biology f collagen part A.* Academic Press. London. 1968 pp. 139–188.

GREENLEE, T K. and R. ROSS, The development of the rat flexor digital tendon, a fine structure study *J Ultrastruct. Res.* 1967 *18* 354–376

GROSS J C. M. LAPIERE and M L. TANZER, Organization and disorganization of extracellular substances The collagen system. In M LOCKE (ed.) *Cytodif ferentiatio nd macr molecula synthesis* Academic Press. New York. 1963 pp. 175–202.

GUSTAVSON, K. H. The chemistry and eactivity of collagen. Academic Press. New York. 1956.

HARKNESS, R. D Biological functions of collagen. *Biol. Rev* 1961 *36* 399–463

HARKNESS, R. D The physiology of the connective tissues of the reproductive tract. In D A. HALL (ed.) *Int. Rev C nnect Tissue Res Vol. 2* Academic Press. New York. 164 pp 155–211

HARKNESS, M L. R. and R. D HARKNESS, The collagen content of the reproductive tract of the rat during pregnancy and lactation. *J Physiol.* 1954 *123* 49?–500

HARKNESS, M. L. R. and R. D. HARKNESS, The distribution of the growth of collagen in the uterus of the pregnant rat. *J Physiol.* 1956, *132* 492–501

HARKNESS, M. L. R. and R. D. HARKNESS, Changes in the physical properties of the uterine cervix of the rat during pregnancy *J Physiol.* 1959a. *148* 524–547

HARKNESS, M L. R. and R. D HARKNESS, Effect of enzyme on mechanical properties of tissues. *Nature* (Lond.) 1959b. *183* 1821–1822.

HARKNESS M. L. R. and R. D HARKNESS, The mechanical properties of the uterine cervix of th rat during involution after parturition. *J Physiol.* 1961 *156* 112–120.

HARKNESS, R. D and M. L. R. HARKNESS The mechanical properties of the uterus compared to those of the birth canal of the rat in pregnancy *Acta physiol. Acad. Sci hung.* 1965. *17* 101–109

HARKNESS, M. L. R., R. D HARKNESS and B. E. MORALEE, Loss of collagen from the uterus of the rat after ovariectomy and from the non-pregnant horn after parturition. *Quart J exp Physiol.* 1956. *41* 254–262.

HARKNESS, M. L. R., R. D HARKNESS and B. E. MORALEE The effect of the oestrus cycle and of hormones of the collagen content of the uterus of the rat. *J physiol.* 1957 *135* 270–280.

HARKNESS, R. D and B. E. MORALEE, The time-course and route of loss of collagen from the rat' uterus during post-partum involution. *J Physiol.* 1956. *132* 502–508.

HARKNESS, R. D and M. A. NIGHTINGALE, The extensibility of the cervix uterus of the rat at different times of pregnancy *J Physiol.* 1961 *160* 214–220

HASHIMOTO J D M. HENDRICKS and L. L. ANDERSON Progestrone and preg-4-en-20 α-ol 3-one in venous blood during the various reproductive states in the rat. *Endocrinology* 1968. *82* 333–341

HEIKKINEN, E. and E. KULONEN Age factor in the maturation of collagen. Intramolecular linkages in midly denatured collagen. *Experientia* (Basel) 1964 *20* 310.

HIRSCH, C. and J GALANTE, Laboratory conditions for tensile tests in annulus fibrosus from human intervertebral discs. *Acta orthop scand* 1967 *38* 148–182.

HISAW F L. and M X. ZARROW The physiology of relaxin. *Vitam. and Horm.* 1950. *8* 151 178.

HODGE, A. G and J A. PETRUSKA, Some recent results on the electron microscopy of tropocollagen structures In S. S. Breese (ed.) *5th Int C gress Electron Microscopy Vol. 2* Academic Press. New York. 1962 pp. QQ-1

HOLTMANN H., G W KORTING, F HAMMERSTEIN K. H. STECHER, M. DU UTTI, P IWANOFF and K. KÜHN Quantitative Bestimmungen der einzelen Kollagenfraktionen der Ha t nach Anwendung von Resochin und Progesterone. N turwissenschafte 1964 *51* 310

JACKSON, D A., Temporal changes in collagen – Aging or essential maturation? *Advanc Biol. Ski* 1965 *6* 219–228.

JEFFREY J J and J GROSS, Collagenase from rat terus. Isolation and partial characterization. *Biochemistry* 1970 *9* 268–273

KOWALEVSKI, K., Effect of pre-pubertal gonadectomy and treatment with sex hormones on body growth, weight of organs and skin collagen of hamsters. *Acta endocr (Kbh.)* 1969 *61* 48–56.

LAROS, G S., C M. TIPTON and R. R. COOPER, Influence of physical activity on ligament insertion in the knees of dogs. *J Bone Joint Su g* 1971 *53A.* 275–286.

LAURENT T C., A. WASTESON and B. ÖBRINK, Macromolecular properties of glycosaminoglycans (Mucopolysaccharides) and proteoglycans. In: A. ENGEL and T LARSSON (eds.) *Thule International Symposia. Aging of Connective and Skeletal Tissues.* Nordiska Bokhandeln. Stockholm. 1969 pp. 65–80

LEPPI, J T A study of the uterine cervix of the mouse. *Anat Rec* 1964 *150* 51–66.

LEPPI, J T and P A. KNUDSON The connective tissue ground substance in the mouse uterine cervix An electron microscopic histochemical study *Anat. Re* 1971 *170* 97–118.

LEVENE, C. J and J GROSS Alterations in state of molecular aggregation of collagen induced in chick embryos by β-aminopropionitril (Lathyrous Factor) *J exp Med* 1959 *110* 771–790

LOBEL, B. L. and H. W DEANE, Enzymic activity associated with postpartum involution of the uterus and with regression after hormone withdrawal in the rat. *Endocrinology* 1961 *70* 567–578.

LOEB, L., V Suntzeff and E. L. BURNS, Changes in the nature of the stroma in vagina, cervix and uterus of the mouse produced by long-continued injections of estrogen and by ad ancing age. *Am. J Cancer* 1939 *35* 159–174

MAIBENCO H. G Connective tissue changes in post-partum uterine invol tion in the albino rat. *Anat. Rec* 1960 *136* 59–71

MATTHEWS, M. B., The interaction of colagen and acid mucopolysaccharides. A model for connective tissue. *Biochem. J* 1965. *96* 710–718

M URER, R. R. and R. H. FOOTE, Uterine collagenase and collagen in young and aging rabbits. *J Reprod. Fertil.* 1972 *30* 301–304

McGAVACK, T H. and K. Y T KAO The influence of age and sex on the soluble collagen, insoluble collagen and elastin of rat tissue. *Exp med Surg* 1960 *18* 104–123

McG VACK, T H and K. Y T KAO Aging in connective tissue. A dynamic process. *J Amer Geriat Soc* 1963 *11* 1024–1032.

MENDOZA, S. A. and R. A. MILCH, Age variations of nominal tensile strength of Wistar rat skins. *Gerontologia* 1964/65. *10* 42–46.

MOHANARADHAKRISHNAN V and N RAMANATHAN Studies on collagen fibers. Part III Strength. *Leather Sci.* 1965 *12* 12–20.

MONTFORT J and R. PEREZ TAMAYO Studies on uterine collagens during pregnancy and puerperium. *Lab Inv st 1961 10* 1240–1258.

MORGAN, F R., The mechanical properties of collagen fibres stress-strain curves. *J Int Soc Leather T ades Chem.* 1960. *44* 170–182.

MUIR, H., Chemistry and metabolism of connective tissue glycosaminoglycans (Mucopolysaccharides) In D A. HALL (ed): *Int. Rev Conn t. Tissue Res Vol. 2*
Anat Rec. 1951 *111* 355–375

MULLER, T The effect of estrogen on the loose connective tissue of the albino rat. *Anat Rec* 1951 111 355–375.

N URMBOLR, A., The structure of collagen. In P A. ZORAB (ed.) Proceedings f symposium of scoliosis, held in London. London 1965 pp. 42

NEUBERGER, A. and H. G B. SLACK, The metabolism of collagen from liver bone, skin and tendon in the normal rat. *Biochem J* 1953 *53* 47–52.

NEUMAN, R. A. and M. A. LOGAN, The determination of hydroxyproline. *J biol. Chem.* 1950. *184* 299–306.

OGSTON, A. G On water binding. *Fed Proc* 1966. *25* 986.

OLSEN, B. R., Electron microscopic studies on colagen. II. Mechanism of linear poly merization of tropocollagen molecules. *Z. Zellforsch.* 1963 *59* 199–215

PIEZ, K. A. and J GROSS, The amino acid composition and morphology of some invertebrate and vertebrate collagens. *Biochim biophys. Acta.* 1959 *34* 24–39

PIEZ, K. A., Soluble collagen and the components resulting from its denaturation. In: G N RAMACHANDRAN (ed.) *Treatise on Collagen. Vol I* Academic Press. New York. 1967 pp. 207–252.

PIEZ, K. A., Discussion. In. A. ENGEL and T LARSSON (eds.) *Thule International Symposia. Aging on co nectiv and skeletal tissue* Nordiska Bokhandeln. Stockholm. 1969 pp 199

PIKKARAINEN J The molecular structures of vertebrate skin collagens. *Acta. physiol. scand.* suppl. 309 1968. pp. 4–72.

PIKKARAINEN J and E. KULONEN, Comparative chemistry of collagen. *Nature* (Lond.) 1969 *223* 839–841

PROCKOP D J and K. J KIVIRIKKO Relationship of hydroxyproline excretion in urine to collagen metabolism. Biochemistry and clinical applications. *A n. Intern Med* 1967 *66* 1243–1266.

RICH, A. and F H. C. CRICK, The structure of collagen. *Natur* (Lond.) 1955. 176. 915–916.

RIGGS, M D and V WRIGHT A rheological study of skin. In R. M. KENEDI (ed.) *Biome hanics and lated bioengineering topics* Pergamon Press. Oxford. 1965 pp. 165–175

RIEHM H., Das Bindegewebe der Vagina während und nach der Geburt. *Arch. Gynäk.* 1951 *179* 145–158.

RIGBY B. J N HIRAI, J D SPIKES and H. EYRING, The mechanical properties of rat tail tendon. *J Gen Physiol.* 1959 *43* 265–283.

ROLLHÄUSER, H., Konstitutions- und Altersunterschiede in Festigkeit kollagener Fibrillen. *Gegenbaurs morph. Jb* 1950. *90* 157–179

ROSENBLOOM J and D J PROCKOP Biochemical aspects of collagen biosynthesis. In J E. DUNPHY and H. W VAN WINKLE (eds.): *Repair and generation.* McGraw-Hill Book Company New York. 1969 pp. 117–135

Kowalevski, K., Effect of pre-pubertal gonadectomy and treatment with sex hormones on body growth, weight of organs and skin collagen of hamsters. *Acta endocr (Kbh.)* 1969 *61* 48–56.

Laros, G S., C. M. Tipton and R. R. Coo er, Influence of physical activity on ligament insertion in the knees of dogs. *J Bone Joint. Surg* 1971 *53A* 275–286.

Laurent T C., A. Wasteson and B Öbrink, Macromolecular properties of glycosaminoglycans (Mucopolysaccharides) and proteoglycans. In: A. Engel and T Larsson (eds.) *Thule International Symposia Aging of Conne tive and Skeletal Tissues* Nordiska Bokhandeln. Stockholm. 1969 pp. 65–80.

Leppi, J T A study of the uterine cervix of the mouse. *Anat. R c* 1964 *150* 51–66.

Leppi, J T and P A. Kinnison, The connective tissue ground substance in the mouse uterine cervix: An electron microscopic histochemical study *Anat Rec* 1971 *170* 97–118.

Levene, C. J and J Gross Alterations in state of molecular aggregation of collagen induced in chick embryos by β-aminoproprionitril (Lathyrous Factor) *J exp Med* 1959 *110* 771–790.

Lobel, B. L. and H. W Deane, Enzymic activity associated with postpartum involution of the uterus and with regression after hormone withdrawal in the rat. *Endocrinology* 1961 *70* 567–578

Loeb L., V Suntzeff and E. L. Burns, Changes in the nature of the stroma in vagina, cervix and uterus of the mouse produced by long-continued injections of estrogen and by ad ancing age. *Am. J Cancer* 1939 *35* 159–174

Maibenco H G Connective tissue changes in post partum uterine involution in the albino rat. *Anat. Rec* 1960 *136* 59–71

Matthews, M. B., The interaction of colagen and acid mucopolysaccharides. A model for connective tissue. *Biochem. J* 1965 *96* 710–716.

Maurer, R. R. and R. H. Foote Uterine collagenase and collagen in young and aging rabbits. *J Repr d Fertil.* 1972. *30* 301–304

McG vack, T H. and K. Y T Kao The influence of age and sex on the soluble collagen, insoluble collagen and elastin of rat tissue. *Exp med S g* 1960 *18* 104–123

McG vack, T H. and K. Y T Kao Aging in connective tissue: A dynamic process. *J Amer Geriat. Soc* 1963 *11* 1024–1032.

Mendoza, S A. and R. A. Milch, Age variations of nominal tensile strength of Wistar rat skins. *Gero tologia.* 1964/65 *10* 42–46.

Mohanaradhakrishnan V and N Ramanathan Studies on collagen fibers: Part III. Strength. *Leathe Sci.* 1965 *12* 12–20.

Montfort J and R. Perez Tamayo Studies on uterine collagens during pregnancy and puerperium. *Lab Invest.* 1961 *10* 1240–1258.

Morgan F R., The mechanical properties of collagen fibres: stress-strain curves. *J Int. Soc Leather T ade Chem.* 1960. *44* 170–182.

UYLDERT, J. E. and O. M. DE VAAL, Relaxation of the rat' uterine ostium during pregnancy. *Acta brev. neerl. Physiol.* 1947. *15*. 49–53.

VERZÁR, F. Veränderungen der termoelastischen Eigenschaften von Sehnenfasern beim Altern. *Experientia*. 1955. *11*. 230.

VERZÁR, F. The aging of collagen. (Simple test of this protein provide objective measurements of the biological age, as distinct from the calender age, of an animal. They also clarify the molecular basis of aging.) *Sci. Amer.* 1963. *208*. 104–114.

VERZÁR, F. Aging of the collagen fiber. In: D. A. HALL (ed.) *I. t. R. Connect. Tissue Res. Vol. 2*. Academic Press. New York, 1964. pp. 244–300.

VERZÁR, F. The stages and consequences of ageing of collagen. *Gerontologia*. 1969. *15*. 233–239.

VIIDIK, A., Biomechanics and functional adaption of tendons and joint ligaments. In: F. G. EVANS (ed.): *Studies the natomy and f ction of bone and joints*. Springer. Heidelberg. 1966. pp. 17–39.

VIIDIK, A., The effect of training on the tensile strength of isolated rabbit tendons. *Scand. J. plast. rec. surg. S. g.* 1967. *1*. 141–147.

VIIDIK, A., Funktion and structure of collagenous tissue. Elanders. Göteborg. 1968a.

VIIDIK, A., An apparatus for th measurements of small tensions. *Experientia*. 1968b. *24*. 861–862.

VIIDIK, A., Elasticity and tensile strength of the anterior cruciate ligament in rabbits as influenced by training. *Acta physiol. scand.* 1968c. *74*. 372–380.

VIIDIK, A., A rheological model for uncalcified parallelfibred collagenous tissue. *J. Biomech.* 1968d. *1*. 3–11.

VIIDIK, A., Chordea tendinea morphology and biomechanics of an unusual collagenous tissue. In: E. SPRING, T. JAUHIAINEN and T. HONKAVAARA (eds.) *Proc. 1 t. Nordic M eting M d. Biol. E g.* Otaniemi. 1970. pp. 200–202.

VIIDIK, A., Simultaneous mechanical and light microscopic studies of collagen fibers. *Z. Anat. Entw.-G sch.* 1972. *136*. 204–212.

VIIDIK, A., Functional properties of conective tissues. I D. A. HALL and D. S. JACKSON (eds.) *Int. Rew. C nect. Tissues Res. Vol. 6*. Academic Press. New York. 1973. pp. 127–216.

VIIDIK, A. and R. EKHOLM, Light and electron microscopic studies of collagen fibers under strain. *Z. Anat. Entw.-Gesch.* 1968. *127*. 154–164.

VIIDIK, A. P. HOLM PEDERSEN and A. RUNDGREN. Some observations on the distant collagen response to wound healing. *Sca. d. J. plast. ec. st. Surg.* 1972. *6*. 114–122.

VIIDIK, A. and T. LEWIN. Changes in tensile strength characteristics and histology of rabbit ligaments induced by different modes of postmortal storage. *Acta. orth. p. ad.* 1965. *37*. 141–155.

VIIDIK, A. L. SANDQVIST and M. MÄGI. Influence of postmortal storage on tensile strength characteristics and histology of rabbit ligaments. *A ta orthop. scand. S ppl.* 1965. *79*. pp. 7–38.

Vogel, G H., Zur Wirkung von Hormonen auf physikalische und chemische Eigenschaften des Binde- und Stützgewebes. Teil 1–4 *Arzneimitt l-Forsch* 1969 19·9: 1495–1503 19 10: 1732 1742 19 11 1790–1801 19:12 1981–1996

Vogel, H. G Beeinflussung der mechanischen Eigenschaften der Haut von Ratten durch Hormone. *Arzneimittel F sch.* 1970. 20, 1849–1857

Vogel, H. G Attempts to correlate tensile strength of skin to solubility of collagen. In H. G Vogel (ed.): *Connective Tissue and Aging* Excerpta Medica, Amstrdam. 1973 pp. 213–218.

Vogel, H. G D Kobalt C W Korting and W Holzmann Prüfung der Festigkeitseigenschaften von Rattenhaut in Abhängigkeit von Lebenalter und Geschlecht. *A ch. klin. exd Derm.* 1970. 239 296–306

Wassermann, F., The intercellular components of connective tissue origin, structure and interrelationship of fibers and ground substance. *E gebn. Anat. Entw -Gesch* 1956. 35 240–333

Warren G H. and R. Fagan Accumulation of dermal mucopolysaccharides in animals following injection of estradiol benzoate. *Proc Soc exp Biol.* 1960 *103* 786–789

Weinstein G D and R. J Boucek, Collagen and elastin of human dermis. *J invest. Derm* 1960. *35* 227–230

Wirtschafter, Z. T and J P Bentley The influence of age and growth rate on the extractable collagen of skin of normal rats. *Lab Invest.* 1962. *11* 316

Woessner, J F Catabolism of collagen and non-collagen protein in the rat uterus during post partum invol tion. *Biochem J* 1962 *83* 304–314

Woessner, J F and T H. Brewer, Formation and breakdown of collagen and elastin in the human uterus during pregnancy and post partum involution. *Biochem J* 1963 *89* 75–82.

Woessner, J F Biological mechanisms of collagen esorption. In. B. S Gould (ed.): *Treatise collagen Vol. 2* Academic Press. London. 1968. pp. 253–330

Wood, G C., The precipitation of collagen fibers from solution. In. D A. Hall (ed.) *Int. Rev Co n ct Tissu Res.* Vol. 2 Academic Press. New York. 1964 pp. 1–28.

Wright D G and D C. Rennels, A study of the elastic properties of plantar fascia. *J Bon Jt Surg* 1964 *46A.* 482–492.

Yoshinaga, K., R. A. Hawkins and J F Stocker, Estrogen secretion by the rat ovary in vivo during the eostrous cycle and pregnancy *Endocrinology* 1969 *85* 103–112.

Zachariae, F Autoradiographic (35S) and histochemical studies of sulfomucopolysaccharides in the rabbit uterus oviducts and vagina. *Acta endocr* (Kbh.) 1958. *29* 118–134

Zarrow M X. and J Yochim Dilation of the uterine cervix of the rat and accompanying changes during the estrus cycle, pregnancy and following treatment with estradiol, progesterone and relaxin. *Endocrinology* 1961 69 292–304

Zimmer, F Die elastischen Eigenschaften des Myometriums und ihre Bedeutung. *Bibl. gynaec* 159 *20* 21–26.

APPENDIX

TABLE

[illegible] trength parameters of uterine horn specimens (mean values ± S.E.M.) from young and old rats

? 1 ** 2P 05 against young control 2P ± 10 TT 2P 0 05 against 1d control

Parameters	No of animal	L_p	Al_{Lc}	$Al_{F_{max}}$	F_{Lp}	F_{Lc}	F_{max}	tan
Dimension ion		mm	mm	mm	N	N	N	N mm
YOUNG								
on ro		3	8 1 3	8 0 3	9 0 1	1 2 0 2	1 5 0 2	1 0 2
Ges at one Days								
1		2**	0 2**	6 0 2**	8 0 1	8 0 2	0 9 0 2*	9 0 5
1	6	0 8	7 6 6	3 0	0 3 0 0**	0 5 1**	8 7 0 2**	0 8 0 2*
1		9 0 7**	10 8 7**	10 0 8**	0.1**	5 8 1**	0 7 0 2**	0 7 0 2**
1		7 5 1**	1 1**	18 9 2 2**	0 2 0**	8 3 0 1**	0 0 1	0 2 0 1**
9 20	8	24 3 2 2*	24 8 2 **	26 6 2 **	0 3 0*	0 3 0**	0 0 0*	0 2 8 0*
7		28 5 5 0*	30 8 8**	31 5 1**	1 0 0**	1 8 0*	0 2 8 0**	0 1 8 0**
Post partum Days								
1	5	20 1 8**	3 1 2**	22 7 1 2**	0 0**	0 1	0 1**	0 3 0 0*
2	2	8 1 3**	13 3 1 8**	6 1 1**	0 5 0 2*	8 5 0 2*	0 9 0 3	0 3 0 1**
3	6	0*	6 0 9	8 8 1 0	1 0 0**	0 1 0 0*	2 0 0**	8 8 1
8		3 7**	3 0 7**	3 **	1 0 0**	0 2 0 0**	0 2 8 8**	0 5 8.1**
1. 1	3	9 0 2**	3 1 2**	2 0 3**	0 2 0 0**	8 0 1**	8 0 1**	1 1 0 2*
15 16	3	5 5**	2 2**	2 2**	4 8 0**	0 5 0.1	0 8 0 1**	1 0
7 20		3 0 4**	5 0 4**	8 3	1**	0 8 0 1**	9 0.1**	1 2 1**
75 30		6 0 1 0	2 1 0	5 8	0 2**	8 2*	0 8 0 2**	1 8 0 1**
OLD								
No of pregnancies								
con rol				6 8	0 3	1 9 8	2 0 5	7
		5 8 0	6 8	6 3 0	6 6 0 1TT	0 9 0 1TT	1.1 0.1TT	1 8 2TT
5		0	6 3	8	0 5 0 1TT	8 8 1TT	0 4 1TT	1 1 0 2TT
		5 8	8 0 9	5 9 9	0 8 0 2	1 2 0 2	1 8 8 3	2 0 5
+5+6	2	6	6 2 0 3		0 4 8 8	0 9 0 1	1 1 0 TT	1 0 2TT

TABLE A II

[illegible] strength parameters of the uterine horn specimens (mean value [illegible]) from young and [illegible] rats
[illegible] 10 ** [illegible] 05 against young control [illegible] 1 VV [illegible] 05 against [illegible] control

Parameters Denomination	No of animals	ε_{Lp}		ε_{Lc}		$\varepsilon_{f_{max}}$		L_p N/mg/mm		L_c N/mg/mm		f_{max} N/mg/mm		tan N/mg/mm	
YOUNG															
Centre	13	27	02	0 33	0 02	0 40	03	[illegible] 1	1 0	11 3	1	1	1	[illegible]	10 [illegible]
Gestat onal Days															
10		0.13	[illegible] 01**	0.18	01**	30	0 **	[illegible]	[illegible]**	[illegible]	0 [illegible]**	7 3	0 [illegible]**	57 1	[illegible]
1		0 13	0 01	.17	**	32	.01**	7	0 7**	7	1.1**	[illegible]	1 0**	70 [illegible]	[illegible]
1	[illegible]	0.13	0 [illegible]**	.18	02**	32	0 [illegible]**	0	0.[illegible]	[illegible]	1	11 0	1 3	[illegible]	[illegible]
1			02**	17	0 03**	23	2**		[illegible]*	[illegible]	0 [illegible]**	[illegible] 1	0 [illegible]**	[illegible] 0	7 0
20		12	0 03**	.18	0 03**	0 23	0 03**	3 [illegible]	1 1	7 [illegible]	1 [illegible]	12 [illegible]	2	[illegible] 1	[illegible] 3
		07	0 02**	09	0 03**	0.13	0 03**	0	0 3*	[illegible]	0 7**	[illegible]	1 1**	[illegible]	19 0
Post-partum Days															
1	5	[illegible] 38	0 03**	0 3	[illegible].03**	40	03*	[illegible] 1	[illegible]	[illegible]	[illegible]*	[illegible]	[illegible]**	38	[illegible] [illegible]**
2		0 28	[illegible] 07	0 30	[illegible]	2	0 [illegible]	[illegible] 3	3 1	[illegible]	3.1	2	[illegible] 1	[illegible]	13 0**
3		07	01	[illegible] 1	0 01**	0 21	03*	1 0	3*		3**	2	3**	1	2 9**
		[illegible]	01**	1	0 03*	22	03**	1 1	0 2*	[illegible]	3**	3 [illegible]	0	18	3 1**
12 13		13	0 **	[illegible] 19	[illegible] 03**	0.23	03**	3	[illegible]	[illegible]	0 3**	7 3	[illegible]**	38 5	5 1**
13 18		0 13	[illegible] 03**	0 18	0 03**	0 [illegible]	0 03**	3	0 3**	[illegible] 3	0 1**	7 5	0 2*	3	1 [illegible]*
20	[illegible]	[illegible] 18	0 03**	0 23	03*	0 33	0 [illegible]	[illegible]	[illegible]*	[illegible]	1 1	12	1 [illegible]	[illegible]	[illegible]
[illegible] 30	3	1	0 08	0.38	[illegible] 08	38	0 [illegible]	[illegible] 0	1.2*	7	1 1	[illegible]	1 0**	33 0	[illegible]*
OLD															
No of pregnancies															
control	[illegible]	28	0 04	[illegible] 38	0 [illegible]	38	0 04	[illegible] 5	[illegible] 7**	7 [illegible]	[illegible] [illegible]**	[illegible] 1	1 0**	[illegible] 1	[illegible] 1
		18	0 01VV	0.30	01VV	0.38	0 01V		0	[illegible] 3	0 7	7 [illegible]	0 [illegible]	[illegible]	[illegible] 3
[illegible]	10	0.18	[illegible] 01VV	.18	[illegible] 02VV	0 27	0.02	3		[illegible] 1	0 [illegible]	7	[illegible]	[illegible] 7	[illegible]
[illegible]		.1	[illegible] 03VV	.17	0 02VV	21	0 03VV	[illegible]	0 7	[illegible]		[illegible]	0 [illegible]	[illegible] 2	7 3
[illegible]	28	18	0 01VV	18	01VV	[illegible] 28	0 01V	3	0 3	3	0 [illegible]	7 3	[illegible]	[illegible]	1

TABLE A III

Tensile strength parameters of the uterine cervix specimens (mean values ± S.E.M.) from young and old rats

*P < 0.10 ** P < 0.05 against young control

Parameters	No of animals	L_{Lg}		ΔL_{LC}		ΔL_{Fmax}		F_{Lg}		F_{LC}		F_{max}		tan	
Denomination		mm		mm		mm		N		N		N		N/mm	
YOUNG															
Control	13			[illegible]		[illegible]	[illegible]	[illegible]	0 [illegible]	[illegible] 7	[illegible]	[illegible]	0 7	[illegible]	0 7
Gestational Days															
[illegible] 10	[illegible]	[illegible]	0.3	[illegible] 7	0 [illegible]	[illegible]	[illegible]	[illegible] 7	0 [illegible]**	0 1	1 [illegible]**	10 [illegible]	[illegible]	17	1 **
13 1		[illegible]	**	[illegible] 7	[illegible] **	7 0	0	[illegible]	0		0 [illegible]	7	[illegible]	[illegible]	[illegible]**
15 16		7 [illegible]	0 [illegible]**	[illegible]	[illegible]**	[illegible]	0 [illegible]**	1 [illegible]	0 [illegible]**	1.[illegible]	[illegible]**	[illegible]	0 [illegible]**	[illegible]	0 **
7 18	[illegible]	[illegible]	[illegible]**	[illegible] 7	0 [illegible]**	10 1	0 [illegible]**	[illegible]	1**	[illegible]	0 1**	0 [illegible]	0 0**	[illegible]	0 1**
[illegible]		10 [illegible]	[illegible]**	1 [illegible]	0 [illegible]**	1[illegible]	[illegible] 7**	0 1	0 0*	0 [illegible]	0 0*	0 [illegible]	0 1**	0	1**
21		12 0	1 [illegible]**	12 [illegible]	1 [illegible]**	1[illegible]	1.1**	0 1	0 0**	0 [illegible]	[illegible] 0**	0	[illegible] 1**	0 [illegible]	0 0**
Post partum Days															
1		[illegible]	[illegible]**	7 [illegible]	[illegible] 7**		[illegible]*	[illegible]	7	[illegible]	0 [illegible]	7 [illegible]	0 7*	[illegible]	0 [illegible]**
[illegible] 3			[illegible]**	7	[illegible] **	[illegible]	0 [illegible]**	[illegible]	[illegible]**	[illegible]	0 [illegible]	[illegible]	0 [illegible]*	[illegible]	[illegible]**
[illegible]		[illegible]	0 [illegible]		7	[illegible]	0.[illegible]	1	0 [illegible]**	[illegible]	0 [illegible]**	[illegible] 1	0 [illegible]**	[illegible] 0	0 [illegible]**
[illegible]	[illegible]	[illegible]	0 [illegible]	7	0 [illegible]	[illegible]	1	1 [illegible]	0 [illegible]**	[illegible] 1	[illegible] 7**	[illegible]	0 [illegible]*	[illegible]	[illegible]*
13 1	[illegible]	[illegible]	[illegible]	[illegible]	0 [illegible]	[illegible]	[illegible]	1 [illegible]	0 [illegible]*	1 [illegible]	0 [illegible]**	[illegible] 0	0 [illegible]**	[illegible]	0 [illegible]**
5 16	[illegible]	[illegible]	1 1	1	1 1	[illegible]	1 1	1 7	0**	[illegible]	[illegible] 0**	[illegible] 7	0.1**	[illegible] 1	0 [illegible]**
1 [illegible]		[illegible]	0 [illegible]	[illegible]	0 [illegible]	1	[illegible]	1.7	[illegible]**	[illegible].1	0 [illegible]**	[illegible]	[illegible]**	[illegible]	0 [illegible]**
[illegible] [illegible]		[illegible]	[illegible]	[illegible]	0 [illegible]	[illegible]	0 [illegible]	1 [illegible]	0 **	[illegible]	[illegible]**	[illegible] 0	0 [illegible]**	[illegible]	1 [illegible]**
OLD															
No of pregnancies															
control	[illegible]	[illegible] 1	[illegible]**	[illegible]	0.[illegible]**	[illegible]	0 [illegible]**	[illegible].1	1 1	[illegible]	1 [illegible]	[illegible]	[illegible]	[illegible]	1 [illegible]
	10	[illegible]	0 [illegible]	[illegible]	0 [illegible]	[illegible]	0.[illegible]	7 1	1 [illegible]	[illegible]	1.[illegible]	1[illegible]	1 [illegible]	[illegible]	1 [illegible]
[illegible]	[illegible]	[illegible] 7	0.[illegible]	[illegible]	[illegible]	[illegible]	[illegible]	[illegible] 7	[illegible]	7 [illegible]	1 [illegible]	[illegible].1	1 [illegible]	7 [illegible]	1.1
[illegible]		[illegible]	0	[illegible]	[illegible] 7	[illegible]	[illegible] 7	[illegible]	1 [illegible]	[illegible]	1 [illegible]	11 1	[illegible]	[illegible]	[illegible]
1+[illegible]	[illegible]	[illegible] 7	0 [illegible]	[illegible]	[illegible]	[illegible]	0 [illegible]	[illegible]	[illegible]	[illegible]	0 [illegible]	11	1 0	[illegible] 0	[illegible]

TABLE A IV

[illegible] strength parameters of the uterine cervix specimens (mean values ± S.E.M.) from young and old rats
P 0.10 ** P [illegible] against young control † P .10 †† P 0.05 against old control

Parameters	No. of animals	ε_{Lg}		ε_{Lc}		ε_{Ymax}		Lg N/mg/mm		Lc N/mg/mm		σ_{Tmax} N/mg/mm		TEM N/mg/mm	
Denomination															
YOUNG															
Control	13	0.36	0.03	0.34	0.04	0.52	0.08	2	1.1	11.6	1.3	26.0	1	50.3	13.2
Gestational Days															
6 10	2	0.27	0.06	0.20	0.07	0.33	0.08*	13	2	18.1	0	21.3	5.6	183.8	26.7**
13 1	5	.22	0.03*	.25	.03*	0.32	0.03**	9.1	.1.2	11.2	1	15	1	91.0	8
15 16	7	0.20	0.02**	0.22	.03**	.38	0.01*	5	0.9**	6.7	1.0**	10.8	1.6**	48	12.7*
17 18		0.06	0.02**	0.13	0.02**	0.24	0.04**	1.	0.3**	3.1	0.3**	1	0.3**	22.6	2.6**
19 20		06	.01**	0.11	0.01**	0.34	.06**	0.8	0.2**	1.8	0.3**	2.8	0.6**	1	2.7**
21	3	06	0.02**	.13	0.01	.40	0.10	0.6	0.1**	1.1	0.1**	2.1	0.2*		1.1**
Post-partum Days															
		0.30	0.03	0.34	0.03	0.45	0.06	5.6	0.8**	7.2	1.2**		0.9**	28.7	5 **
2 3		0.21	.04	0.27	0.04	.33	0.03**	5.5	1.7	7.8	1.6	10.6	2.9*	38	1
5		0.17	0.04	0.48	0.08	0.77	0.18	.3	1.6**	5.9	2.0**	7.3	2.2**	37.1	18.1
		0.28	0.1	.46	0.18	0.53	0.08	3.2	0.9**	4	.9**	6.2	2.1**	19.7	6.1**
13 1	3	0.22	0.03*	.34	0.03*	0.38	.02**	8	1.1**	8.1	1.1	3.8	1.9**	52.8	3*
15 16	2	.13	0.01**	0.23	.01**	28	0.01**	3	1.6**	5	1.6*	6.8	3.3**	3.6	26.8
17 20	5	0.23	0.03	0.27	0.03	0.2	.06	5.8	0.9**	7.4	1.3**	11.2	2.0*	87.2	13
25 30		0.30	0.06	0.38	0.04	0.7	0.04		0.7**	6.7	0.5**	12	0 **	50.0	8.6*
OLD															
No. of pregnancies															
0 control	6	0.25	0.03	0.38	0.02	0.43	0.06	5.7	1.0*	7.3	1.1**	11.2	2.0**	82.5	10.7
	10	0.31	0.02	0.38	0.02††	0.7	04	6.6	0.6	8.8	0.7	13.8	1.1	80.3	3.9
	6	0.38	0.04	33	0.04	0.40	04	5.3	8	6.8	0.5	8.7	0.6	49.3	5.8
8		0.38	0.04	0.33	0.04	0.38	03		1.	7	1.2	9.8	1.7	51.8	11
4+8+8	23	38	0.02	0.33	0.02	0.42	0.03	5.9	0	7.7	0.5	10.8	0.7	54.7	3.3

TABLE A

Tensil trength parameters f he vaginal wall specimens (mean values ± S E M.) from young and old rats

P ?P 0? against young control † ?P 1 †† ?P 0 05 against old control

P ameter	No f animal	ε_{L_B}		ε_{L_E}		$\varepsilon_{T_{max}}$		T_{L_B}		T_{L_E}		T_{max}		tan	
Denomination		mm		mm		mm						N		N/mm	
YOUNG															
on rol	1	1	0 6	18 2	0 6	19 5	0 8	1	1	1 5	0	2 1	0 2		0 1
Ge onal days															
		19 2	1 1	20 5	1	23 2	1 2**	0	0 1	7	1**	1 0	0 1**	0	0 0**
1		22 1		23 3	0 8**	26 2	1 0*		0 0**	0 8	1**	0 9	1	0 2	0
1	6	23 6	1 **	24 2	1 5	28 3	1 **	0	0 1**	0 8	1	0 8	**	0 3	0 0**
		24 8	**	25 4	1 **	28 7	1 **	3	1		0 1**	0 6	1**	0 1	**
1 20	8	27 3	2*	28 1	1 1**	30 7	1 0*	5	1**	0 6	0 1**	0 9	1	2	0
1		28	1 5*	27 3	1 **	30	1 5	3	0 1**	0	0		1**	0 2	0 1**
Post partum days															
		24	2	26	**	27	2		0 1	0 7	1**	8	2	0 3	0 1
	7	20	6	21 8	0 5**	22 2	8**	0	0 2*	8	0 3	1 2	0 2**	0	0 1
	5	18 2	1	18	1	8	3	6	0 1**	9	1	1	0 3**	6	2
9	3	22 1	0 **	22 9	**	24	0 7**	0	0 1	1 0	2	1	3		0 1
13	2	20 7	0 2**	21	0 1**	23	1 0**	5	0**	8	0 1**	1 3	1**	0	0 0**
1		22	1 2*	23 1	1 8*	2	8*	0 3	0 2	8	2**	1 4	1		0 0
1 20	5	20	1	21	1 0**	23	1 **	0 8	2	1 1		8	8	8	
OLD															
No of pregnancies															
control	8	1 2	1 2	8	1 2	19 8	1 3	1	2	3	3		0 **	1 0	0
	15	18 1	0	8	0	20 1	8	1	0 2	1 6	0 2	1 2	2†	0 8	1
		9	1	1 8	1	20 6	1	1 1	1	1	0 2	1 8	0 ††	8	1
4		22 5	2 6	23 2	2 6	25 3	2	0 8	2†	1 2		1 8	0 ††	0 5	0 2†
1-6	28	19 0	6 7	18 7	6.7	21 0	8		0	1 5	8 1	2 0	2††	8	8 1

TABLE A-VI

Tensile strength parameters of the vaginal wall specimens (mean values ± S.E.M.) from young and old rats

*P 10 ** P .05 against young control. †P 0.10 ††P 0.05 against old control

Parameters	No of animals	ε_{LB}		ε_{LE}		ε_{Tmax}		σ_{LB} N/mg/mm		L_E N/mg/mm		σ_{Tmax} N/mg/mm		tan N/mg/mm	
Denomination															
YOUNG Control	13	0.26	.02	0.25	0.02	0.36	.02	5	0.7	12.7	0.9	15.6	1.1	67.9	5
Gestational Days															
6 10	3	0.12	.02**	0.18	0.02**	0.32	0.04	.8	1	8.3	2.3	13.3	2	3.0	23.6
13		.1	.02**	0.19	0.03	0.39	0.06				1.0**	13	1.7**	56.1	7**
15 18	6	0.1	0.01**	0.17	0.01**	.28	.02*	7.2			1.2*	1	1.4*	82.1	7.8
17 18	7	0.11	0.02**	0.18	0.02**	0.32	0.02	5	1.8	7.7	2.2**	11.1	2.3**	63.4	18.3
19 20	3	0.17	0.02	21	0.02	0.33	0.03	10.4	3.2	13.4	3.7	18.4	2	99.9	38.5
21		.1	0.03	0.17	0.03**	0.21	0.04	4.8	1.7**		2.1**	7	2.0**	50.0	25.1
Post-partum Days															
1		0.11	0.02**	0.17	0.02**	0.39	.02**	3.7	1.0**	6.8	1.3**	8	1.1**	7.7	8.1**
2 3	2	0.15	0.01	0.20	.02	0.39	0.01**	5	.4**	5.5	0.7**	11.8	.3**	75.2	5.2
4 5	8	0.10	0.01**	0.13	0.01**	0.38	0.03**		0.2**	6.5	0.5**		1.0*	70.3	8.0
7 9	3	0.1	0.02*	0.10	0.02**	0.36	0.03**	7.4	0.7	18.4	0.**	13	1.0*	8	8
12 13	2	12	0.03**	0.15	0.03**	0.28	0.01**	8	0.1	18.0	0.7**	17.2	1.4	51.0	1
1 16	3	.13	0.03**	0.15	0.04**	0.37	0.02**	7	2	10.8	2.7	19.8	1.2	100	17.3
17 20	5	.13	.03	.21	0.03	0.35	0.03	7.3	1.7	9.9	1.9	16.7	3.5	78.5	13.2
OLD															
No of pregnancies															
0 control	8	0.16	.02	0.21	0.02	0.33	.03	7	1.8	11.1	1.2	16.6	1.1	74.9	6.7
4	15	.15	0.02	0.20	0.03	.28	0.02	8.8	0.7	8	0.8	12.4	1.1††	61	5
5	9	0.18	0.02	0.21	0.02	0.27	0.03	6.8	7	9.3	0.8	12.0	1.2††	74.8	8.1
6		0.16	0.02	0.13	0.02††	0.23	0.01††	5.7	0.9	9.8	1	15.8	1.0	82.0	6.9
4+5+6	8	0.15	.01	0.19	0.01	0.37	0.01†	6.3	0.4	9.1	0.8	13.7	0.8††	72.0	3.6

TABLE A-VII

Tensile strength parameters of the pubic symphysis (mean values ± S E M) from young and old rats

*P < 0 10 ** P < 0 05 against young control   †P < 0 10 †† P < 0 05 against old control

Parameters	No of animals	Al_{L_p}		Al_{L_r}		$Al_{F_{max}}$		F_{L_p}		F_{L_r}		F_{max}		tsr	
Dependent for		mm		mm		mm						N		N/mm	
YOUNG															
Control	13	0 17	0 03	0 25	0 04	1 30	0 1	2 1	0 3	0	0 5	1 0	1 0	24 2	3 8
Gestational Days															
8 10	3	0 33	0 08*	0 48	08	1 82	0 10	3 4	0 6	5 4	5*	13 3	0	25 6	7
13 1	7	0 40	0 09**	0 35	0 13**	1 34	19	5	0 5**	6	1 2*	12 0	1 0	19 0	2 3
15 16	8	5	0 08**	0 60	0 08**	1 64	0 10	4 5	0 6**	6	0 6**	1 1	2 1	13 5	1 1**
17 18	5	40	0 10*	0 35	0.12**	1 31	07	3 5	0	5	6	13 3	1 2	18 2	2 1
19 20	8	0 32	0 03	0 3	0 04	1 30	0 09	3 6	0 6**	5 3	0 8	12	1 0	18	1 6
21	2	0 7	0 08**	0 50	0 08	1 0	0 04	4 1	0 6**	1	0 7**	1 8	3	15 9	2 1
Post partum Days															
1		0 31	0 13	0 48	15	1 90	0 14	2 8	0 6	5	0 8	12	8 8	15 8	6**
2 3		0 40	0 08*	0 54	0 09**	1 88	0 31	2 5	8	5 6	0 **	12 2	0 7	18 8	2 3
5	6	29	0 07	0 3	0 08*	1 79	29	2 8	5	8	0 6	12 5	1 4	19 6	3 5
7 8	3	0 30	0 08	0 54	19	1 50	1	3	0 1**	8 3	1 2*	13 8	0 5	15 5	2 **
13 13	2	0 0	0 08**	0 57	08**	1 39	0 80	1	1**	7 3	1	16 1	1 6	31 2	0 8
1 16	5	0 35	0 08*	8	0 10*	1 65	33	2 7	0 7	5 7	1 1	14 3	8 7	23 3	1
17 20	6	0 25	0 07	0 31	0 08	1 28	5	3	1 0	6 9	1	13 3	1 0	28 2	9
25 30		0 26	03*	0 34	0 04	1	08	2	0 3		0	14	1 0	23 1	2 7
OLD															
No of pregnancies															
0 control	6	0 13	02	0 29	0 03	1 65	30	1 8	0 3	5 6	0 1	13 9	1 2	20 9	1 9
	13	0 12	0 03	0 31	0 06	2 15	0 1	1 7	0 3	3 6	0 5††	13	0 6	23 1	2 1
5	9	0 06	0 02	0 18	0 43††	2 22	0 23	1 3	0 2	3 7	0 6††	15 6	0 9†	26 1	3 1
6		0 36	0 10	42	12	2 36	0 3	3 3	0 9	5 5	0 8	12 0	0 5	17 3	6 5
5+6	25	0 13	0 02	11	0 03	2 23	0 11	1 8	0 3	3 9	0 ††	13 9	0 5	22 5	1 7

TABLE A-VI I

Tensile strength parameters of the posterior cruciate ligaments (mean values ± S E M.) from young and old rats

*P <.10 ** P <.05 against young control

Parameter	No of animals	ΔL_B		ΔL_F		$\Delta L_{F_{max}}$		F_{L_B}		F_{L_F}		F_{max}		tan	
Dimension in		mm		mm		mm		N		N		N		N/mm	
YOUNG															
Control	12	[illegible]	[illegible]	1.11	0 06	1.19	[illegible]	13	0 7	18 [illegible]	1 0	21 3	0	1 0	1 [illegible]
Gestational Days															
[illegible] 10	[illegible]	0 79	[illegible]**	0 [illegible]	0 [illegible]**	1.02	[illegible]**	1	0 [illegible]	1 [illegible]	0 [illegible]	22 [illegible]	[illegible] 7	[illegible]	2 1
13	[illegible]	[illegible]	0.05	1.11	[illegible]	1 13	0.10	13 [illegible]	0.[illegible]	18 [illegible]	[illegible]	18 [illegible]	0 [illegible]**	[illegible] 0	1 [illegible]
13 18	[illegible]	[illegible]	03	1.07	0.03	1.16	[illegible]	13.	[illegible]	20.1	1.1	23 [illegible]	0.[illegible]	[illegible]	1 1
18		0 [illegible]	0.04	1 [illegible]	[illegible]	1.18	[illegible]	13 [illegible]	1 3	19 [illegible]	1.1	21.6	1.3	[illegible]	1.4
1 20		79	0 [illegible]	0.[illegible]	[illegible]**	[illegible]	03**	13 1	7	18 [illegible]	[illegible]	20 [illegible]	0 [illegible]	41 [illegible]	1
21	1	0 [illegible]	[illegible]	1.04	[illegible]	1.16	[illegible]	1 [illegible]	1.3	17 [illegible]	2 0	19 7	1.7	[illegible] 3	2.[illegible]
Post partum Days															
		[illegible]	[illegible] 13	[illegible]	0.13	1.1	0 07	[illegible] 2	1.0**	13.1	1 [illegible]	1	[illegible]**	[illegible] 2	4
	3	[illegible]	[illegible]**	0.[illegible]	0.04**	[illegible]	[illegible] 04**	7 2	0 [illegible]	11	0 [illegible]**	15 2	0	[illegible]	2**
			.10	0 [illegible]	[illegible]	1.07	[illegible]	11 7	1 1	18 3	1.[illegible]	22 7			[illegible]
[illegible]	3	0.78	0 [illegible]	[illegible]	0.07**	1.12	0 04	18 5	[illegible] 7**	23 [illegible]	1 **	23 1	[illegible]**	33 [illegible]	[illegible]
12 13	3	0.[illegible]	[illegible]**	0.77	0.07**	1.00	0.10	13 3	1 [illegible]	18.1	0 6	24 0		5	7
14 18		1 00	[illegible]	1.11	0.08	1.[illegible]	0.02	13 [illegible]	0.[illegible]	15 [illegible]	0	19 7	1	[illegible] 7	2.1
1 20	[illegible]	[illegible]	[illegible]	1.00	0 08	1.08	[illegible]	12	1.2	16	1 0	20	1 3	1 1	3 1
25 30		1.17	[illegible]	1.76	[illegible]	1 34	0 08	13	2 2	19.1	2 2	21			
OLD															
No of pregnancies															
control	7	1 [illegible]	0.08	1.18	0 08	1.23	[illegible]	11	1 [illegible]	16 [illegible]	1 [illegible]	18 7	1 **	37 [illegible]	1 0*
	14	[illegible]	[illegible]	1.08	0 08	1 12	08	10	0	1		15 1	[illegible]	[illegible]	1
[illegible]	[illegible]	1.04	[illegible]	1.1	0 08	92	0 08	10	[illegible]	14.3	1	1	0 [illegible]	[illegible]	1 3
[illegible]		[illegible]	0 [illegible]	1 [illegible]	[illegible]	1 07	0 09		1.1	1 3		1 0	[illegible]	[illegible]	[illegible]
[illegible]	37	0 [illegible]	[illegible]	1.08	0 04	1 14	[illegible]	10 3	0 [illegible]	13	[illegible]	18 3	0	[illegible]	1.1

TABLE A IX

[illegible] strength parameters of muscle tendon specimens (mean values ± S.E.M.) from young and old ra[illegible]

[illegible] ** 2P 0 0[illegible] against young control 2P 10 [illegible] 2P 05 against 1d control

Groups	No of animals	L_B		L_C		$\varepsilon_{T_{max}}$		L_B N mg/mm		L_C N mg/mm		T_{max} N/mg/mm		tan N/mg/mm	
Denomination YOUNG															
[illegible]l		07	00	08	0 1	15	0 01	90	7 [illegible]	11 [illegible]	1 [illegible]	234 1	25	2600	275
[illegible] [illegible]al Day															
1		09	02	0 10	0 01	0 1	0 01	139 [illegible]	21 3**	208 0	[illegible] 6**	[illegible]	33 [illegible]	411	[illegible]
		07	01	[illegible]	01	0 12	0 1	[illegible] 5	10.1	1 1 [illegible]	20 [illegible]	211 [illegible]	30 1	2981	613
1[illegible]	6	07	0 01	08	0 01	0 15	02	[illegible]	11 7	118 [illegible]	19	18 1	35 3	2058	190
1[illegible]		08	00	10	[illegible] 01	0 15	0 01	[illegible]	11 [illegible]	1 [illegible]	1 [illegible]	211	27	2793	397
[illegible]		08	00	08	08	[illegible] 12	0 01	58 3	[illegible]**	92 [illegible]	1	1 [illegible] 2	28 **	1855	281
1	3	06	0 01	07	0 01	0 12	0 02	[illegible]	1 [illegible]**	[illegible] 1	[illegible] 0*	1 [illegible] 3	1 1	115	607
Post partum Days															
		06	01	08	0 1	13	0 01	1 1	3 6**	137 7	16 2	188 [illegible]	22 [illegible]	2081	37
		0 08	0 01	10	01	[illegible] 18	1	80 [illegible]	[illegible] 0	133 [illegible]	7 [illegible]	192 [illegible]	19 3	[illegible]	11
0		0 07	[illegible] 01	[illegible] 04	0 01	0 13	03	76	7 [illegible]	127 [illegible]	1	201 1	[illegible]0 2	2548	333
[illegible] 10	3	06		0 08	01	0 1	1	71	[illegible]	109 [illegible]	33	180 3	27 [illegible]	1160	116
OLD															
No f regardless															
control		08	01	.1	0 1**	0 18	0 01	11 [illegible]	25	173 0	1 3	37	[illegible]	1752	58
	1	18	1		01	0 21	01[illegible]	76 1		106 [illegible]	[illegible] 1	72	13 [illegible]	1183	60
		1	0	13	1	[illegible]	[illegible] 62	75 [illegible]	[illegible]	108 [illegible]	1 [illegible]	67	1 [illegible]	106	82
		12	03	1	72	0 22	02	103	13 1	138 7	7 [illegible]	183 [illegible]	23 [illegible]	1383	139
[illegible]	2[illegible]	1	1	1	0 01	0 21	0 01[illegible]	79 4	[illegible]	111	[illegible]	1 2	1	1135	47

TABLE

Tensile strength parameters of skin specimens (mean values ±) from young and old rats

1 2P 05 against young control 2P 10 †† 2P 05 against old control

Parameters	No of animals	L_B		L_C		εT_{max}		L_B N/mg/mm		L_C N/mg/mm		σT_{max} N/mg/mm		TAE /mg/mm	
Measured on															
YOUNG															
Control	1	0 22		27	02	44	08	27 8	2.3	40 1	3 6	61		2 7	27 1
Pregnant Days															
		23	01	0	0 02	0 39	0 01	35 6	2 9*	53 3	6**	73 6	2 7*	335 6	17 4**
		25	02	.31	02	0 6	0 03	36 5	2 3**	51	2 0**	7	3 6	377	22 7*
15 16		21	01	23	0 01	0 40	0 04	27 0	2 8	30	6	43 2	3	264 8	25 6
17 18	6	0 22	0 02	18	0 02	0 38	0 03	31 1	3	6 2	8	67 6	5.5	282 6	17 9
20	12	0 21	0	0 25	0 01	0.38	0 03	31 6	3 2	8 2		70 1	5	357 9	28 8*
21		0 28	03	30	0 01	0 41	0 04	33 7	2 7	41 1	3 7*	44 0	2 2	703	20 5
Post-partum Days															
1	5		0 1	28	0 01	37	00	34 0	3.8	34 6	1 7**	62.3	5	34 5	55 3
		0 22	01	0 28	0 02	0 37	08	28 1	6	44 2	10 8	58	20 5	338 2	88 7
13 1		25	01	0 29	1	0 41	0 03	38 5	1	38	2 8	51 3	6.0	278 9	15 2
1 [illegible]		22	01	36	02	0 40	0 02	31	3 0	34 0	3		7	302 3	36 6
[illegible] 38		0 24	01	30	0 02	1	0 03	36.8	1 **	58 0	1	77 0	4 7*	3 3	71 3
OLD															
Pregnant															
Control		0 20	01	1	01	.38	0 02	36	3 1	81 9	**	100	12 2**	424 8	55 1*
		74	0 01	33	0 0	0 38	01	40		74 6	3	86	6	377 6	24 2
	[illegible]	28	0 0 ††	34	0 01††	0.	02	34		64	1	74 0	6	325	33 3
		23	0 02	0 33	03	0 48	0 02	35.1	2		6	86	1	361 5	28
		24	01††	34	††	0 3	0 01	37 7	2 8	7	2	82		350 0	10.3

TABLE A XI

Thermal isometric contraction versus time (mean valu ± S E M) of tail t dons in young and old rats
 2P 0 10 2P 0 05 ag inst young ontrol
▼ 2P 0 10 ▼▼ 2P 0 05 ag inst old control

(Tension is normalized to mg tendon fresh w ight per cm tendon l gth)

	No of animals	Tension Maximum 10 ⁶N	Time mi
YOUNG			
Control	13	1430 ± 50	0 58 ± 0 04
Gestational Days			
6 10	3	1750 ± 50	0 87 ± 0 07
13 14	9	1579 ± 63	0 67 ± 0 06
15 16	9	1658 ± 102	0 67 ± 0 05
17 18	7	1620 ± 96	0 77 ± 0 05
19 20	13	1574 ± 74	0 72 ± 0 03
21	4	1610 ± 60	0 70 ± 0 06
Post partum Days			
1 2	3	1593 ± 137	0 80 ± 0 12
3 4	4	1895 ± 86	0 8 ± 0 10
5 7	3	1800 ± 120	0 67 ± 0 07
12 15	9	1760 ± 50	0 87 ± 0 06
16 20	9	1760 ± 80	0 91 ± 0 08
25 28	4	1910 ± 70	0 9 0 10
LD			
N f p egrar e			
0 ontro	3	376 ± 77	2 52 ± 0 16
	15	4147 ± 92▼▼	2 94 ± 0 16▼▼
	10	4018 ± 107▼	3 13 ± 0 22▼▼
		3759 ± 76	3 90 ± 0 52▼▼
4 5	3	3972 ± 60▼▼	3 23 ± 0 14▼▼

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 418

From the Department of Medical Pharmacology
University of Uppsala, Sweden.

CARBONIC ANHYDRASE HISTOCHEMISTRY
RITICAL STUDY OF HANSSON S COBALT PHOSPHATE METHOD

by

Gudmar Lönnerholm

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 418

From the Department of Medical Pharmacology
University of Uppsala Sweden.

CARBONIC ANHYDRASE HISTOCHEMISTRY
A CRITICAL STUDY OF HANSSON'S COBALT PHOSPHATE METHOD

by

Gudmar Lönnerholm

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 418

From the Department of Medical Pharmacol gy
University of Uppsal Sweden

CARBONIC ANHYDRASE HISTOCHEMISTRY

A CRITICAL STUDY OF HANSSON s COBALT-PHOSPHATE METHOD

By

Gudmar Lönnerholm

INTRODUCTION

The first published histochemical technique for demonstration of carbonic anhydrase was that of Kurata (1953) It has however been found to give unsatisfactory results by most workers (Hausler 1958 Fand et al 1959 Pearse 1968)

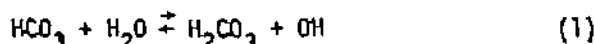
Häusler (1958) described a histochemical method with sections floating on the medium which was slightly modified by Waldeyer and Häusler (1959) The validity of this method has been accepted by some (Korhonen and Korhonen 1965 Leder 1971) and denied by others (Hursta kallio et al 1960 Arnold 1966 Muther 1972 Churg 1973) The long incubation times 20-120 minutes are a serious drawback since dif fusion of the enzyme or the reaction product may take place Inhibi tion of the staining requires millimolar concentrations of acetazol amide an inhibitor with a K_i of about 10^{-8}M This makes it doubtful if the inhibitor indeed acts in a specific manner and precludes its use as a check of the specificity of staining

Hansson (1967) modified Häusler s method by adding phosphate to the medium This changes the composition of the reaction product and

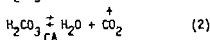
allows much shorter incubation times 1 15 min A further and most important difference is that specific inhibitors of carbonic anhydrase at micromolar concentrations abolish the staining

In Hansson s method sections are floated on the surface of a freshly prepared incubation medium containing $NaHCO_3$ $CoSO_4$ H_2SO_4 and KH_2PO_4 Increasing amounts of cobalt and phosphate accumulate in active sections during the incubation (Hansson 1967) However the exact nature of the cobalt phosphate complex has not been clarified By treatment with $(NH_4)_2S$ it is visualized as a black precipitate probably containing CoS The deposition of cobalt and phosphate as well as the visible staining is inhibited by low concentrations of acetazolamide (Hansson 1967)

As discussed by Hansson the dehydration of HCO_3 to CO_2 and OH is the basis of the histochemical staining Continuous local OH formation at sites of carbonic anhydrase activity causes deposition of the basic cobalt phosphate complex The processes responsible for the alkalinization can be written



$$HCO_3 + H_2O \rightleftarrows H_2CO_3 + OH \qquad (1)$$



$$H_2CO_3 \underset{CA}{\rightleftarrows} H_2O + \overset{\uparrow}{CO_2} \qquad (2)$$

The loss of CO_2 is the essential reaction which forces reactions 1 and 2 to the right resulting in a production of OH No staining occurs if the loss of CO_2 is prevented by keeping an atmosphere of CO_2 above the medium with the floating sections

The phenomena close to the fluid surface are the important ones since the catalyzed staining reaction can occur only where there is CO_2 loss to the air The sections remain unstained if they dip under the surface during the incubation (Hansson 1967)

Hansson s method has been used in a number of studies (Hansson 1968 Laurent et al 1969 Pesetsky 1969 Lauwers et al 1970 Rosen 1970 1972 a b Musser and Rosen 1973 a b Rosen and Friedley 1973 Bhattacherjee 1971 1972 Lönnerholm 1971 1972 1973 1974 Lönnerholm and Ridderstråle 1974) Distinct and consistent staining patterns

were reported by these authors Further available biochemical data were found to correlate well with the histochemical staining

The method has been criticized by Muther (1972) however He questions its specificity on the basis of a number of experiments some of which will be discussed in the course of the present paper Others of his findings are dealt with in a counter critique by Rosen and Musser (1972) Lightfoot and Cassidy (1973) have also argued in favour of the validity of the method

In our laboratory data have accumulated which strongly support the idea that Hansson s method is indeed specific for carbonic anhydrase activity These findings are reported here

GENERAL METHODS

TISSUE PREPARATION

Fre h tis ues were used f biochemical ssay Ti ues to be tudied hi tochemically wer fro en by imme i in isopentane cool d with liquid nit gen with or without p vious fixation They we tor d in small plasti bags t 70°C f days week bef us

The fixative was 2 5 % glutaraldehyd in 0 1 M ph phat buff (pH 7 4) The glutar ldehyd was prepared from 50 % t ck s lution by on stage va uum di till tion and th purity f th di till t w checked by ultravi l t pectroph tometry (And rson 1967) Ti ue blocks (maximum thickn 4 mm) were immers d in th fixative for 16-20 h t 4°C They we then rinsed in 0 2 M ucros 0 05 M phosphate buffer (pH 7 4) to remove excess fixative Some ti sues w perfused with fixative i_ vivo (ee b low) with o without sub eque t fixation by immersion

Human kidney tissue was obtained from fresh dono kidneys prepared f r transplantation by pe fusion with cold Ring -dextran type f sol tion They wer sto ed t 4°C Th ti sue prepar tion tarted within 24 hr Furth d tail on th se kidneys re given by Lönnerh lm (1973)

Pro tate tissue was taken f om 8 Wistar ts (300 g) b ain and rvical spinal co d ti sue f om 4 mal Spr gue-Dawley ts (300-500 g) and kidneys from femal Wi t ats (250-300 g) All t we e f d tandard t pellets and tap wate d libitum Th y w anestheti ed with pentob rbital odium (Mebumal® ACO) 40 mg/kg i j cted i tr periton ally The tissue t be tudied was perfused i_ vivo with aline for 5 min The purpo was t emove th rythrocyt which contai large amounts f th nzyme When fixed ti ues wer desired the perfusion with aline was f llowed by perfusion with th fixative for 10 mi

Spinv d gfi h <u>Squalus enthias</u> were captur d at the Swedish Atlantic co st in October and transported to th laborat ry in a tank with oxygen-bubbl d eawater They were used within 24 hrs of d livery One male and two females weighing 1 5 - 3 kg were studied They v re ana thetized with MS 222[R] (Sandoz) 100 mg/L of tank water A polyethylene tube was int oduced int th dorsal aorta and the caudal part of the animal w s perfused with blood isotonic 3 3 % NaCl f r 5-10 min Kidney ctal gland and skel tal muscle tis ue which all appeared blood fr e after the perfusion we then dissect d They were eithe homog nized for biochemical assay or frozen with or without pr vi s fixation for histochemical study

HISTOCHEMICAL STAINING PROCEDURE

Se tions were cut in cryostat at 20°C

S ctions f fixed tissue 8 µm or thick r were collected on cold 0 2 M sucrose 0 05 M ph sph te buffer (pH 7 4) They wer transferred to the incub ti n medium within 3 min

S tions f unfixed tissue could not be handled in thi way ince they disintegr t t th contact with th fluid The efore 15 µm thick se tions were fr e e-d i d for 1 hr at 0 03 mm Hg and 50°C and then incub ted Thes s ctions could b stored f r some days b f re use

When thinner se tion of eith fixed or unfixed tissues were desi ed they had to b supported by piece of Millipore[R] filter (TH WP 25 µm thick pore size 0 45 µm Millipor Filter Corporation Bedford Mas USA) Freshly cut s ctions wer thawed ont piece of filter To ascertain good adhesion they w r kept f r few min tes t room temper tur b fo the incubation

The staining procedur of Hansson (1967 1968) was used with only minor modifications Sections w re flo ted n the surface of a medium containing $CoSO_4$ 1 75 mM H_2SO_4 53 mM KH_2PO_4 11 7 mM and $NaHCO_3$ 157 mM Thi concentr tion of KH_2PO_4 allows short incub tion times of 1 15 min and wa used thr ughout this tudy wh not therwis tated Th volume f the medium was 57 ml It was used immediately aft r the freshly prepared bicarbonate s lution (0 75 g $NaHCO_3$ in 40 ml f distilled w t) had been added to th oth r compon nts If drugs were added t the medium they were inc rp rated into the bicarbonat s lution b fore mixing

The pH f th medium i 5 8 immedi tely aft r mixing but increases due to f rmation and l of CO_2 int the ai Th pH at th surf ce of the medium duri g the incubation i not known However an upp r limit is set by th pH f medium when it has (ft r ev ral hours) eq ilib ated with the i ; this v lue is 8 5 (Han son 1967)

Se tions not upported by Millipo e[R] filte s tend to disintegrate in th slightly eff rves ent medium but wer prote ted b re by addition of the non-ionic det rgent Tween 20[R] t th medium in a final concentr tion f 1/100 000 (v/v)

Afte the i cub tion the se tions we rinsed in saline buffered with phosphate (0 67 mM pH 5 9) They w then tran ferred to the freshly p epared bl ckening olution 0 5 % $(NH_4)_2S$ in distilled water

Bef r us this lution was filte ed through a filter pape where CoS had b en depo ited

All sol tions w kept t room temperatur (22 24 C) The s c- tions we transferred between th sol tions by glass rod P tri dishes (diamet r 9 5 cm) w re used for all s lutions Th choice of ves l f the incub tion medium i f importanc in th ize f the s rf ar influen the r t f CO_2 l from th medium i to the ai

The different t ps f the taining pro edur can b summarized

1) Float section on the incubation medium immedi t ly ft r mixing Mak e that th sections do not dip under th surf ce of the medium
2) Fl t sections on th buff red line for 3 min
3) Fl t ections on the blackening solution f 3 min
4) Rinse f r 1 min i three ccas iv line di hes
5) Captur ctions on slide and 1 t them dry
6) If desired count retain with g hematoxylin and eo ine
7) Dehyd at ction through graded concentr tions f thanol xylen and mount with Canada bal am.

When ections on Millipo [R] filters re used th staining proce- dur has to be omewhat modified

Ad 1 Allow the ctions n thei filte to equilib te with the me- dium f 10 min unde CO_2 tmo phere Thi was h re accompli hed by blowing 100% CO_2 ove th medium with th fl ti g se tions by means f an inve ted funn l pl ced over the P tri di h Thi p e- vent escape f CO_2 from th medium, which i the basi f the re- action When the CO_2 tmospha i removed the incubation t rts Th ections must b on the top f th filt during th incuba- tion

Ad 2 3 4 Immerse th tions on th i filt to secure dequat ex posur f th tions to th l tion

Ad 5 D hyd t and mount immedi taly aft r th final rinsing

Ad 7 Dehydr te in 95% thanol ab lute n-propanol xylene and n-pro- panol (1:1) xylene and mount with Canada b l am. Th filt r be- come t ansparent during th dehyd ation which make it pos ibl to mount th ections t g th with thei supporting filte

INHIBITORS

Ethoxz lamid (Upj hn) tazolamide methazolamid Cl 13850 (Ameri- can Cyanamid) lfanilamid (Chemapol) benzthiazide (Pfize) and chl rothiazide (Me ck Sharp and Dohme) were used without further pu- rification They we di lved in w te ; f benzthiazid it was ne- cessary to add 2 mol s f NaOH t ch mol f inhibito

DETERMINATION OF ENZYME CONCENTRATIONS

The con entr tion f purifi d enzyme was measured pect ophotometri- ally as using an extin tion coefficient $A_{280}^{1\%}$ cm^{-1} f 16 3 and 17 8

f r HCA B and HCA C respectively (Gibbons and Edsall 1964) Apo-HCA B was assumed to have a siril r value as HCA B Th mol cule weight was taken to b 30 000 f r ll enzymes test d

BIOCHEMICAL ASSAY OF CARBONIC ANHYDRASE ACTIVITY

Fr h tissues wer weighed and homogenized by a Teflon[R] plunger in glas tube with 9 part f 0 25 M ucr se (dogfish tissues) or distil led wat r + 1 mM EDTA (rat prostat) The wh le homogenat s were used Th activity of the homogenates and of purified enzymes was determined by th changing pH method of Philpot and Philpot (1936) as d s ribed in d tail b Ma en et al (1954) CO_2 i bubbled at constant rat through the rea tion ves el containing phenol red A standard amount f carbonate buffer is added which rais the pH and cause th indicator to change col r Th hydr tion f CO_2 utr li e the added buf f r b se and causes th indicator t r turn to the original (acid) co l r within a ertain time On enzyme unit i d fined as the ctivity which reduces this time by h lf The a tivity of sampl was calculated from 1 2 determinations on each of 3-4 dilution of the sampl The as ays were performed t 0 2-0 4°C

I_{50} DETERMINATIONS

Th ctivities of the ulfonamides used (Tabl IV) were d termined as their I_{50}-v lues t 0 2 0 4 C by the changing pH method according to Ma n t al (1954)

Pi c s of human kidney co tex which had been stor d at 70°C we thawed weighed and homogenized with 9 parts f 0 25 M ucros Aft r centrif g tion t 100 000 x g and 4°C f 60 min th supernatant was removed and used f r th assays Th amount f supernatant corresponding t 1 enzyme unit w d t rmin d Twice that amount was then used and th concent tion f drug yi ldi g a r ction time corresponding t that f 1 enzyme unit was taken as the I_{50}-v lu Each d t rmination of I_{50} in Table V is the v r g of 4-6 runs

PHOTOMETRY

An obj tiv but semi-quantitative comp rison f the histochemical staining i the various tions was attempt d by means of a photomet r Fig 1 S tions f human kidney co tex which had b n t from the sme piec f ti ue and wer of approximat ly th same size we ed (f r det il e Table VI) This tissue contains truct r s with ma kedly diff rent enzyme tivitie which gives an uneven dist ribution f th taining d po it i th ections Th ref re th photomet was set t meas the light which pas d through the wh l of the tions th than thro gh a small andomly l cted part Fo thi p rpo the ape ture of th photomet r was t to barely exceed the f th ctions and was then left unchanged during the whole meas i g proc dur

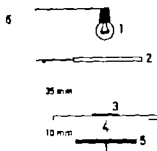


FIG 1 Construction of the ph tomet r (not drawn to cale)
1) Stabili ed light urc
2) Opalesce t s reen
3) Section on it lid
4) Adjustabl pertur
5) Selenium barrier-layer cell with low resistanc load The current gener ted by th ph tocell is ecorded by a galvanomet
6) Met l box (black) Its upper part can b removed

Test sections incubated in the presence f various sulf namides wer compared with un tai ed and fully stained (uninhibited) tions Th photomete gener ted tronge current with unstained than with fully stained ections sinc l s light pas ed through th latt The difference between the means of th unstained (n=4) and th f l ly tained (=8) ections was calcul ted Parti l taining could then b calcul ted as per entag f thi diff renc How diff rences i photometer eadings relate to diff ences in taining intensity is not known in detail However the purpos f the present expe ime t was t compare numb f tions i semi-quantitative way Th ppar tus used ppear t b adequate for thi limited purpo

RESULTS AND DISCUSSION

POSITION OF THE STAIN WITHIN THE THICKNESS OF THE SECTIONS

Rat kidney sections 8 20 μm thick were incubated in the standard medium After the final rinse they were embedded in paraffin Transections of the original sections were then cut and mounted The *staining deposits were found to be located only at the upper surface* of the sections i e the surface which had been in contact with the air during the incubation Fig 2 As studied in detail by electron microscopy (Lönnerholm and Ridderstråle 1974) the stain penetrates with decreasing intensity only a few μm of tissue leaving the lower part of the sections unstained Free floating sections and sections supported by Millipore[R] filters were similarly stained As described in Methods free floating sections are floated on all solutions whereas sections on Millipore[R] filters are floated on the incubation medium but *immersed in the rinsing and blackening solutions* Thus, the uneven distribution of the staining is not an effect of the procedures following the incubation i e rinsing and blackening

The present findings on the distribution of the staining within the sections support the idea (see Introduction) that the phenomena at the surface of the incubation medium are the important ones As one leaves the surface CO_2 loss rapidly becomes rate limiting instead of dehydration of HCO_3

The findings further indicate that pH-gradients caused by enzyme activity are established within the sections rather than in the medium This should explain why local OH accumulations resulting in deposition of the basic cobalt phosphate complex can be maintained at enzyme sites in spite of the effervescence of the medium during the first minutes after mixing

Huther (1972) calculated that loss of CO_2 and not dehydration of HCO_3 is the process which determines the rate of alkalinization of the medium as a whole (my italics) He felt that this argued against



FIG 2 T ansection of 20 µm thick free floating action f fixed r t kidney stained for carbonic anhydras activity Incubation time 15 min Note that th taining deposits re located only t th urface in contact with th air during th incub tion Arrows how rythrocytes inside ves l, wher thos ituated at the surface r tai ned and th e in the lower part f th section ar unstained x 1000

the mechanism of the staining outlined above However the present findings show that the analysis of the staining phenomena cannot rest on considerations concerning changes in the bulk of the medium The same conclusion was drawn by Posen and Musser (1972) who found that a pH gradient rapidly develops in the medium (in the absence of sections) after mixing with a more alkaline pH close to the surface

Since the histochemical staining reaction takes place in a micro milieu which is not easily accessible for a detailed chemical analysis other kinds of experiments were designed for the present study of the specificity of the method

PURIFIED ENZYME PREPARATIONS COMPARED BIOCHEMICALLY AND HISTOCHEMICALLY

Native enzymes Low activity (HCA B) and high activity (HCA C) human erythrocyte carbonic anhydrase isoenzymes were prepared by ion exchange chromatography according to Nyman (1961) The activity of these purified enzymes was measured biochemically by the changing pH-method (see Methods) and in histochemical model experiments In the latter 5 µL droplets of serial dilutions (1 2) of the enzymes were deposited on small pieces of Millipore[R] filter by an Eppendorf[R] micropipette and

TABLE I The activities of purified HCA B and HCA C preparations as measured biochemically and in histochemical model experiments

Enzyme preparations	Biochemical method		Histochemical method	
	enzyme units/nmole of enzyme	relative activity HCA C / HCA B	smallest amount of enzyme producing detectable staining (pmoles)	relative activity HCA C / HCA B
1 HCA B	1 7		23	
HCA C	9 8		5 1	
		5 8		4 5
2. HCA B	1 3		26	
HCA C	9 3		4 0	
		7 2		6 5

allowed to dry in the air for 10 min The pieces of filter were then incubated separately without any previous equilibration under CO_2 The incubation time was always 9 min After the final rinse in saline the stained spots on the filters (about 5 mm in diameter) were observed by the naked eye without mounting on slides In this way the lowest con centration of the enzyme giving just visible staining was titrated In the critical region 2 3 spots were read for each concentration

Both isoenzymes produced staining in the histochemical model experi ments the minimal detectable amounts were about 25 pmoles for HCA B and 4 5 pmoles for HCA C (Table 1) Thus HCA C was roughly 5 5 times more active than HCA B When samples of the same enzyme preparations were assayed biochemically HCA C was about 6 5 times as active as HCA B (Table 1)

In the histochemical test and in the changing pH method the enzyme operates under different conditions with respect to temperature (22 24 and 0°C) substrate (HCO_3 and CO_2) and pH range (5 8-8 5 and 7 4 10 0)

At first glance it therefore seems surprising that the same relation between the activities of the enzymes is seen in the two tests However this relation is rather constant also in kinetic measurements where the conditions have been varied to the same extent (Magid 1968 Wistrand et al 1974) It is only at pH values of 10 or more that the activity of HCA B approaches that of HCA C as a catalyst of the CO_2 hydration reaction (Khalifah 1971)

Modified enzymes Each molecule of carbonic anhydrase contains one zinc ion which can be reversibly removed from both HCA B and HCA C (Lindskog and Nyman 1964) The metal free apoenzyme has no catalytic activity No important change of the protein structure seems to accompany the loss of the zinc ion (Lindskog and Malmström 1962 Wistrand and Rao 1968 Brewer et al 1968) A number of divalent metal ions bind to the active site of the apoenzyme The addition of Zn^{2+} activates the enzyme completely and Co^{2+} restores about half of the activity but the Cu^{2+}-complex is inactive After removal of these ions by dialysis catalytic activity can usually be restored by addition of Zn^{2+} indicating that denaturation of the enzyme has not occurred (Lindskog and Malmström 1962) In the present experiments metal free apoenzyme and Zn^{2+} Co^{2+} and Cu^{2+}-enzymes were prepared from HCA B according to Lindskog and Nyman (1964) The biochemical assays showed that two experiments (1 2 in Table II) yielded apoenzymes with low residual activity However the addition of Zn^{2+} restored less than half the activity indicating that inactivation of some enzyme had occurred The third apoenzyme preparation showed considerable residual activity probably due to incomplete removal of the zinc ion Reactivation with Zn^{2+} was complete

The smallest amount of enzyme which produced detectable staining was titrated histochemically as described above The relative activities were then calculated as percentage of that of the native enzyme to enable comparison with the biochemical findings The histochemical and biochemical data were found to agree quite well (Table II) with a correlation coefficient r for the two sets of data of 0 91 (p<0 01)

TABLE II The activities of various enzyme preparations as measured biochemically and in histochemical model experiments

Enzyme preparation	Activity as percentage of native enzyme Biochemical method[a]	Histochemical method
1 Apoenzyme	1	< 5[b]
Zn^{2+} reactivated enzyme	22	16
2 Apoenzyme	1 4	3
Zn^{2+} reactivated enzyme	43	52
3 Apoenzyme	22	27
Zn^{2+} reactivated enzyme	110	84
Co^{2+} enzyme	56	84
Cu^{2+} enzyme	6	10

[a] The changing pH method was used as de cribed in Method section except for experiment no 3 wher the assays were made in a stopped flow apparatu (s e Wi trand et 1 1974 f r details) at 25 C and pH 7 03 sing CO_2 as the substrate

[b] More concentr ted enzyme not a ilable

According to Lindskog and Nyman (1964) Co^{2+} rapidly combines with metal free HCA B Since the histochemical medium contains this ion some reactivation of the apoenzyme by formation of Co^{2+} enzyme during the incubation could be expected However the data of Table II show that no or little reactivation occurred possibly because the composi tion of the histochemical medium is unfavourable

In summary the present findings show that the catalytic activity of various enzyme preparations as measured biochemically agrees well with the ability of the enzymes to produce staining in histochemical model experiments This strongly supports the idea that the demonstra tion of carbonic anhydrase with Hansson s method depends on the cata lytic activity of the enzyme

UNCATALYZED AND CATALYZED STAINING

When inactive tissue sections are incubated long enough the sponta neous alkalinization of the medium due to the uncatalyzed reaction $HCO_3^- + OH^- + CO_2$ causes visible staining Therefore the staining is specific and enzyme-dependent only if it occurs earlier than that of the uncatalyzed reaction Hansson (1967) found that by changing the concentration of phosphate in the medium one could influence the time of appearance of both the catalyzed and uncatalyzed staining

With the phosphate concentration used here 11 7 mM inactive sec tions remained unstained until after 20-25 min of incubation a dif fuse staining appeared This was seen in sections of a tissue lacking carbonic anhydrase i e dogfish skeletal muscle and in human kidney sections inactivated by heat see Table III The staining was diffuse-ly distributed except for the nuclei which generally appeared some-what more stained than the other structures This uncatalyzed staining was not influenced by addition of the inhibitors acetazolamide or eth-oxzolamide (Table III)

Active uninhibited human kidney sections became stained already after 1 2 min of incubation With increasing concentration of inhibi tor in the medium the incubation times for visible staining were pro longed until they were similar to those of inactive sections With 1 µM acetazolamide some tubular cells of the kidney sections became stained after 10-15 min of incubation (Table III) thus indicating considerable but not complete inhibition Virtually total inhibition as evidenced by diffusely distributed staining appearing only after 20-25 min of incubation was seen with 10 µM acetazolamide and 1 µM ethoxzolamide (Table III) a few experiments with 10 times higher con centrations of these inhibitors gave similar results (data not shown)

The findings show that the incubation time should not exceed 15 min when the present high concentration of KH_2PO_4 in the medium is used However with structures containing large amounts of the enzyme it might be necessary to use even shorter incubation times to prevent diffusion artifacts These are easily recognized as areas of diffuse

TAPLE III The histochemical staining of tissues where the enzyme activity is high (kidney) lacking (muscle) or inactivated by sulfonamides or heat

Tissue	Inhibitor in the medium		Incubation time (min) 3	6	10	15	20	25	30
Dogfish muscle[b]									
	no inhibitor						(+)	+[a]	+
	acetazolamide	10 µM					(+)	+[a]	+
Heat inactivated *human kidney cortex*									
	no inhibitor						(+)	+[a]	+
	acetazolamide	10 µM					(+)	+[a]	+
	ethoxzolamide	1 µM					(+)	+[a]	+
Human kidney cortex									
	no inhibitor		+	+	+	+	+	+	+
	acetazolamide	1 µM			(+)	+	+	+	+
		10 µM					(+)	+[a]	+
	ethoxzolamide	1 µM					(+)	+[a]	+

positive - negative ( ) weak and inconsist nt staining Each point represents s tions from at least 5 incub tions II 7 mM KH_2PO_4 in Il *incubation media 8 µm thick f a floating sections of fixed* tissues war used Unfixed kidney ti ue w boiled for 10 min frozen and cut to give heat inactivated s ctions (8 µm thick)

Some sections we e unstained [b]Homogenates of dogfish mus l do not contain d te tabl enzyme activities s e Table VII As shown in Fig.11 B the mus l fibers ar ina tive but one capill ri s may how weak cti-vity This capillary staini g was neglect d he

gray black staining surrounding the intensely stained sites The most suitable time must be found for each structure

Two conclusions can be drawn from the present findings

1) The ability of the sulfonamides to inhibit the staining in active sections is due to their activity as inhibitors of carbonic anhydrase and not to unspecific interactions with the medium since they do not influence the uncatalyzed staining in inactive sections

2) The staining pattern in active tissues for example the human kidney is not caused by non-specific accumulation of the precipitate at some tissue components since the staining caused by the uncatalyzed reaction in sections inactivated by the sulfonamides is diffusely distributed

THE DEPOSITION OF PHOSPHATE (^{32}P) IN THE SECTIONS

The purpose of these experiments was to confirm the findings described in the previous paragraph by studying the catalyzed and uncatalyzed histochemical reactions by a quantitative method Hansson (1967) found a parallel increase with time in the deposition of ^{60}Co and ^{32}P in active sections There was a concomitant increase in the intensity of the staining Muther (1972) also studied the ^{32}P uptake into sections and extended the incubation period to 60 min He found a very slow initial uptake into kidney sections in the presence of acetazolamide 10 µM However the uptake rate increased after 30-40 min of incubation He thought that this could be explained by an unspecific interaction of the inhibitor with the medium possibly by complexing Co^{2+} Since it was felt that Muther s interpretation is not correct another purpose of the present experiments was to clarify the mechanism behind the uptake rates observed by him

^{32}P labeled ortophosphate with approximately 10^{10} cpm/mole of phosphate was included in the medium at the usual concentration Sections of human kidney cortex (8 µm thick free floating) were incubated in the usual way At timed intervals one section was removed from each batch of incubation medium The sections were rinsed in the usual

TABLE III The histochemical staining of tissues where the enzyme activity is high (kidney) lacking (muscle) or inactivated by sulfonamides or heat

Tissue	Inhibitor in the medium		Incubation time (min) 3	6	10	15	20	25	30
Dogfish muscle[b]									
	no inhibitor						(+)	+[a]	+
	acetazolamide	10 µM					(+)	+[a]	+
Heat inactivated human kidney cortex									
	no inhibitor						(+)	+[a]	+
	acetazolamide	10 µM					(+)	+[a]	+
	ethoxzolamide	1 µM					(+)	+[a]	+
Human kidney cortex									
	no inhibitor		+	+	+	+	+	+	+
	acetazolamide	1 µM			(+)	+	+	+	+
		10 µM					(+)	+[a]	+
	ethoxzolamide	1 µM					(+)	+[a]	+

= positiv - negative ( ) = weak and inconsistent taining Each point r p esents sections from at least 5 incubations 11 7 mM KH_2PO_4 in all incubation media 6 µm thick f floating sections of fixed ti sues w used Unfixed kidney tiss e was boiled f r 10 min frozen and cut to give heat inactivated s ctions (6 µm thick)

Some s tions wer unstained [b]Homogenates f dogfi h mus l do not contain d te tabl enzyme activities see Tabl VII As shown in Fig.11 B the mus l fibers r inactive b t some capillaries may show weak cti-vity Thi apill ry taining was neglected h re

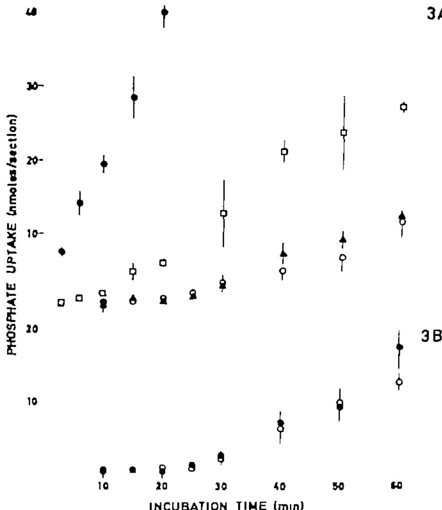


FIG 3 Depo ition of phosphate in se tions f human kidney cortex Mean value ±1 S E St ti tical unit ections A) tive ti ue (fixed) ● no inhibit r (n=4); □ tar lamide 1 μM (n=3); O acetazolamide 10 μM (n=6); ▲ thoxzolamide 1 μM (n=6) B) ina tive ti ue (unfixed boiled f r 10 min) ● no inhibit (n=5); O= cetazolamide 10 μM (n=5) All section i A wer cut from th ame piece of ti sue ll ctions i B from another Th tions in A were omewhat small than tho i B (about 0 5 and 0 6 cm² r spe tively)

for 16 hrs rinsed in sucrose for 2 hrs and frozen (see Method section for further details)

Tissue blocks were thawed weighed and homogenized with distilled water + 1 mM EDTA The whole homogenates were assayed by the changing pH method The activity of unfixed and fixed renal cortex was 195 ± 20 6 and 16 4 ± 3 4 enzyme units/g wet weight respectively (means of 3 samples ± 1 S E) Thus the activity of the fixed tissue was 8 4 % of the unfixed similar to Muther s (1972) value for glutaraldehyde-fixed tissue

20 µm thick sections (n 50) were cut and floated on 0 2 M sucrose + 0 05 M phosphate buffer (pH 7 4) at 22 24°C for 10 min They were then homogenized and assayed as described above Control sections cut from the same piece of tissue were homogenized immediately after cutting In each experiment the activity of the floated sections was calculated as a percentage of the controls The unfixed sections were found to retain only 7 2 ± 1 6 % of their original enzyme activity whereas the fixed ones retained full activity 118 ± 14 7 % (means of 3 experiments ± 1 S E)

These data would explain why fixed kidney sections show the same or a more intense staining than unfixed ones the partial inactivation of the enzyme caused by the fixative is compensated for by the seemingly complete immobilization of the enzyme in the sections

COMPARISON OF DIFFERENT SULFONAMIDES AS INHIBITORS OF CARBONIC ANHYDRASE AND AS INHIBITORS OF THE HISTOCHEMICAL STAINING

A comparison would seem to be difficult because the pH range covered is different in the histochemical and changing pH methods 5 8-8 5 and 7 4 10 0 respectively However both methods mainly utilize the pH range 6 5 9 where the activity of the sulfonamides is rather constant

TABLE IV. CHEMICAL AND PHYSICAL PROPERTIES OF SULFONAMIDES WITH SUMMARY OF THEIR EFFECTS IN BIOCHEMICAL AND HISTOCHEMICAL TESTS

NAME AND M.W.	STRUCTURE	(pK_a)[a]	ETHER PARTITION COEFF[b]	INHIBITORY EFFECT: BIOCHEMICAL TEST (SULFANILAMIDE=1)	INHIBITORY EFFECT: HISTOCHEMICAL TEST (GRADED FROM TO ++++)[c]
ETHOXZOLAMIDE 258	C_2H_5O … $C-SO_2NH_2$	8.1	140	500	++++
ACETAZOLAMIDE 222	$CH_3-C(O)-N(H)-$ … $-SO_2NH_2$	7.4 (9.1)	0.14	110	+++
METHAZOLAMIDE 236	$CH_3-C(O)-N=$ … CH_3-N … $-SO_2NH_2$	7.2	0.62	100	+++
BENZTHIAZIDE 432	CH_2SCH_2 … Cl … SO_2NH_2 … O_2	7.4		200	+++
CHLOROTHIAZIDE 296	Cl … SO_2NH_2 … O_2	6.7 (9.5)	0.04	5.7	++
SULFANILAMIDE 172	H_2N … SO_2NH_2	10.4	0.15	1	
CL 13850 278	CH_3-C-N … $-SO_2NHC(CH_3)_3$	7.5 (10.2)		NO EFFECT	NO EFFECT

[a] FROM MAREN (1964); MAREN ET AL (1960). THE pK OF BENZTHIAZIDE WAS SUPPLIED BY PFIZER.

[b] THE RATIO OF CONCENTRATIONS IN ETHER AND PHOSPHATE BUFFERED SALINE (pH) FROM HOLDER ET AL (1960)

[c] THE BIOCHEMICAL DATA WERE CALCULATED FROM THE DATA OF TABLE . THE HISTOCHEMICAL GRADING SUMMARIZED THE FINDINGS SHOWN IN FI AND TABLE VI

(Taylor et al 1970 a) At a more acid pH the activity of all sulfonamides decreases equally probably due to titration of a group on the enzyme Above pH 9 the activities of the individual sulfonamides are affected differently and this could possibly influence the results with the changing pH method However Maren and Wiley (1968) found that I_{50} values of a large number of sulfonamides measured with this method in barbital buffer pH 7 4 7 9 are similar to those in carbo nate buffer pH 7 4 10 0 Therefore the differences in pH range bet ween the histochemical and changing pH methods as used here should not influence the activity of the sulfonamides sufficiently to preclude a comparison of their relative potency in the two methods

Seven sulfonamides with widely different structures were selected for this study see Table IV

The activities of different sulfonamides have been found to vary with the form of enzyme tested and particularly large differences have been observed between the high and low activity (with respect to CO_2) forms (Wistrand 1965 Taylor et al 1970 b)

It was therefore felt necessary to determine the inhibitory activi ties of the sulfonamides against the enzyme of the same tissue as tes ted histochemically The activities were measured at 0 2 0 4°C using the changing pH method Inhibitor and enzyme were equilibrated for different times with and without the presence of CO_2 Human renal cortex was used as enzyme source

The results are seen in Table V The sulfonamides were found to in hibit the human renal enzyme with I_{50} values similar to those previous ly found for the high activity forms of the human (Wistrand 1965) and dog (Maren and Wiley 1968) erythrocytes This would agree with the evi dence accumulated so far (see Wistrand et al 1974) that the major part of the activity of the human kidney depends on an enzyme which is simi lar to if not identical with the high activity human erythrocyte form HCA C Five minutes of equilibration of enzyme and inhibitor in the presence of CO_2 increased the activities 2-4 fold and apparently so for all sulfonamides (cf 1st and 2nd columns of Table V) However incubating the thiazides with the enzyme in the absence of CO_2 increa

TABLE V The inhibition of carbonic anhydrase from human kidney cortex by different sulfonamides at 0 2 0 4°C

Drug	SEI		EI			
	Equilibration time (min)		*Preincubation time (min)*			
	0	5	1	5	30	60
Ethoxzolamide	0 011	0 003			0 004	
Acetazolamide	0 036	0 010			0 011	
Methazolamide	0 014	0 005			0 012	
Benzthiazide	2 6	1 3	0 086	0 033	0 006	0 006
Chlorothiazide	17	10	0 80	0 33	0 21	0 21
Sulfanilamide	2 4	1 4			1 2	
Cl 13850	a	a			a	

Th inhibition i exp e ed as I_{50}- lues i th concentr tion f inhibito (µM) that duced the nzyme activity by half (se Methods)

SEI enzyme and inhibit were added in th t rd to the CO_2-bubbled reaction v el Th timed eaction was tarted immedi tely (1st column) aft r 5 min f equilib tion (2nd column)

EI enzyme and inhibito wer preincub ted t id the eaction ves l t 0°C They were then added t the CO_2-bubbl d ve l and the rea tion was tarted immediately

a No inhibitory eff t t 50 µM

sed their activities approximately 100 fold (cf SEI and EI data for chlorothiazide and benzthiazide) No such effect was seen for the other sulfonamides Why CO_2 (or HCO_3) prolongs the time of equilibra tion between the thiazides and the enzyme has not yet been settled (see Lindskog et al 1971) However as shown by Maren and Wiley (1968) *the thiazides can be made to equilibrate rapidly with the enzyme both* in the presence (SEI) and absence (EI) of CO_2 if temperature is raised to 37°C

In the histochemical test the intensity of the staining of fixed human kidney cortex sections was estimated either by the eye in the light microscope (Fig 6) or by photometry (Table VI)

Fig 6 summarizes the microscopic findings Ethoxzolamide was found to be the most active drug inhibiting the histochemical staining completely even at the lowest concentration used 0 5 µM Aceta zolamide and methazolamide were equally active and slightly more so than benzthiazide Chlorothiazide was clearly less active than benz thiazide but more active than sulfanilamide The control sulfonamide Cl 13850 had no activity at 50 µM Fig 7 shows an example of how the sections differed in staining intensities when incubated with diffe rent concentrations of chlorothiazide

The estimation of the staining intensity of the sections by photo metry agreed well with the subjective 6-min observations (Table VI)

The relative order of potency in the histochemical reaction corresponds well with that in the biochemical test particularly so if the inhibitors were allowed to equilibrate with the enzyme outside the reaction vessel (the EI data of Table V) This is understandable since in the histochemical reaction the temperature 22 24°C favours inhibition equilibrium In some control experiments where the sec tions had been preincubated with the inhibitors without presence of bicarbonate (see legend to Fig 6) the results were similar to those of the standard experiments of Fig 6

In a preliminary study (data not shown) unfixed human kidney sec tions (15 µm thick freeze-dried) were tested To obtain the same degree of inhibition somewhat higher concentrations of the sulfonamides were required than with fixed sections The relative poten cies of the sulfonamides were unchanged however

In summary then as shown in Table IV the relative potencies of seven widely different sulfonamides as inhibitors of the histochemi cal staining were found to agree well with their relative potencies as inhibitors of carbonic anhydrase

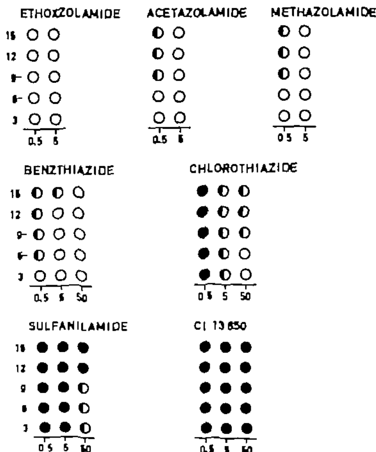


FIG 6 Eff t of lfonamide on the histochemical taining f ections f fixed human kidney cortex; light mi ro copi tudy Concent ation f drug in th medium (μM) on the x-axis Incub tion time (min) on the y-axi ● no di rnibl inhibition ◐ p rtial inhibition ○ t tal inhibition 8 μm thick free floating ections which had been cut from the same piece f kidney tissue were incubated with each inhibito t th diff rent concentr tions Since thes experi ments we e epeated three times each point shown ep esents i gle ection from 3 different incubations

With acetazolamid benzthiazid and chl rothiazid sections we ls tained after preincubation with th inhibito (dat not hown) Thi was don by floating e tions f 5 min on buff red (pH 7 4) sucro l tion (22 24°C) which contained the ame con ntr tion f inhibit as the incub tion medium

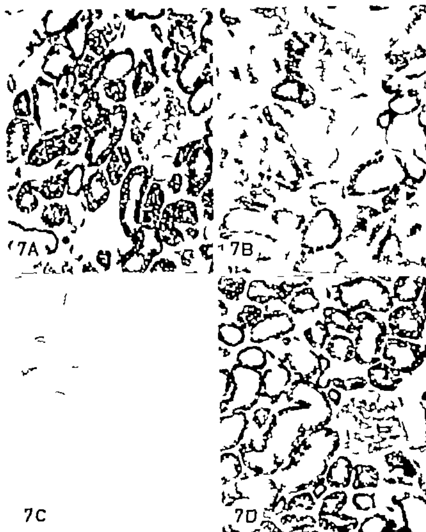


FIG 7 Effect f diff ent concentr tion (A = 0 5 µM B 5 µM C 50 µM) of chlorothiazide on th taining of human kidney ec- tions D shows contr l tion incub ted without inhibitor The s se tions a the sme as used in Tabl VI x 180

TABLE VI The effect of sulfonamides on the histochemical staining of sections of fixed human kidney cortex as measured photometrically

Drug	Concentration of drug in the medium (µM)		
	0 5	5	50
Ethoxzolamide	1 (0 2)	3 (2 5)	
Acetazolamide	11 (0–17)	1 (0 2)	4 (0 7)
Methazolamide	4 *(0–9)*	2 *(0 3)*	4 *(2 7)*
Benzthiazide	57 (41–67)	6 (0 10)	1 (0 3)
Chlorothiazide	113 (102 121)	60 (57–62)	1 (0 3)
Sulfanilamide	95 (88–103)	111 (102 119)	69 (47 107)
CI 13850	117 (97 140)	96 (78 116)	132 (122 145)

Th staining intensity of the ections i expressed as per entage f uninhibited contr ls (f d tail ee Methods) Mean v lues f 4 c tions and ang re hown The incub tion time was lways 6 min 8 µm thick f ee floating tions we used and 11 s tions wer cut from th ame piece f ti sue

ADMINISTRATION OF ACETAZOLAMIDE IN VIVO

Sodium acetazolamide (Diamox[R] parenteral Lederle) dissolved in 0 9% NaCl was injected into the tail vein of male Sprague-Dawley rats weighing 300–400 g In each experiment 4 rats were used One rat was given saline only and the others 20 40 and 100 mg/kg body weight of the drug respectively The injected volume was always 1 ml/kg body weight 30 min after the injection the kidneys were perfused (for details see Lönnerholm 1971) with saline for 3 min (4 experiments) or with fixative for 5 min (2 experiments) and then rapidly removed and frozen Kidney sections were cut to include both cortex and medulla Two sets of sections were prepared from each animal one with free

floating sections (15 µm thick) and another with sections on Millipore[R] filters (4 or 15 µm thick) Each set of sections was incubated in one dish and one section was removed after 2 4 6 9 and 12 min respectively Sections on Millipore[R] filters were incubated without previous equilibration under CO_2 Free floating sections fixed as well as unfixed were freeze-dried and then incubated

As seen in Fig 8 A D the injected acetazolamide inhibited the staining in the kidney sections with a clear dose-response relationship 20 mg/kg (Fig 8 B) gave a somewhat delayed and weakened staining as compared to *saline controls* (*Fig 8 A*) *40 mg/kg* (*Fig 8 C*) gave still weaker staining and 100 mg/kg (Fig 8 D) inhibited all staining in some sections Unfixed kidneys (photos not shown) showed a similar dose-response relationship

The present findings agree with Hansson s (1967) report of delayed and weak staining in mouse kidney and gastric glands after 20 mg/kg acetazolamide intravenously Bhattacherjee (1971) found inhibition of the staining of rabbit eye tissue after injection of 10 mg/kg of the same inhibitor

We found it essential to handle the sections so as to avoid contact with any fluid before the timed incubation started Thus when the sections were *floated on buffered sucrose for 10 min before* transfer to the incubation medium even 100 mg/kg of the drug failed to produce clearly visible inhibition (Fig 8 E) the procedure had no effect on the staining of uninhibited control sections These findings are not surprising since acetazolamide is a reversible inhibitor of the enzyme (Taylor et al 1970 b) They might explain why Muther (1972)

FIG 8 Eff t f in vivo acetazolamid on the taini g f r t kidneys Fixed 4 µm thick ections supported by Millipo R filt rs Incubation time 12 min A aline control B 20 mg/kg C 40 mg/kg D 100 mg/kg cetazolamid *E was cut from the same pi c of kidney tissue* a D but the section was pr i cub t d for 10 min in buff red ucro e bef re stai i g All phot g aphs ar from the outer cortical zon 180 (A) x 160 (B-E)


8A
8B
8C
8D
8E

did not see any affect of injected acetazolamide 20 mg/kg on the staining of rat kidney sections the inhibitor was probably lost from the sections before the incubation since they were first collected in saline (unfixed sections) or floated on cold acetone (fixed sections)

CORRELATION BETWEEN HISTOCHEMICAL AND BIOCHEMICAL FINDINGS IN VARIOUS TISSUES

A number of tissues known to contain widely different amounts of carbonic anhydrase were chosen for a comparison between biochemical data and histochemical findings They have been summarized in Table VII

Mawson and Fisher (1952) and Leiter (1964) reported high carbonic anhydrase activities in homogenates of the dorsolateral rat prostate but only low activities in the ventral prostate This strikingly un even distribution of the enzyme was confirmed here biochemically and histochemically The dorsolateral rat prostate showed heavy staining of the whole glandular epithelium already after 1 3 min Fig 9 A In the ventral prostate only scattered stained sites were seen even af ter incubation for 15 min Fig 9 B

All vertebrates except sea going fishes seem to have abundant renal carbonic anhydrase (see Maren 1967 a) Its importance for urinary acidification is suggested by experiments with inhibitors of the enzyme (see Maren 1967 b 1969) Histochemically high enzyme activity is found in the proximal and distal tubules as well as in the collec ting duct in the mammalian kidney (Lönnerholm 1971 1973 Rosen 1972 a) Single species of aves reptilia and amphibia have also been studied histochemically and clear enzyme activity was found in various parts of the kidney tubules (Rosen 1972 a Lönnerholm and Ridderstrå le 1974)

In homogenates of the kidney of the spiny dogfish (Squalus acanthias) no or very low enzyme activity is found Table VII This correlates with an inability to vary the urinary pH and with a lack of effect on the urinary composition of acetazolamide in vivo (Hodler et

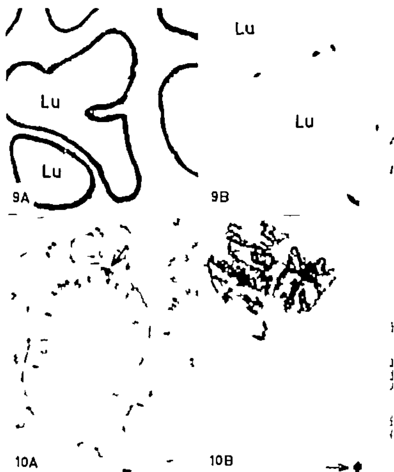


FIG 9 Do 1 te 1 (A) and ve t 1 (B) r t pro tat Fixed 8 µm thick f es fl ting ections Incub tion time 3 min (A) 15 min (B) L = glandul lumen Not intens unif rm taining f th pithelium in A In B only few tained ites in th th rwis inacti pithelium en The glandul tructu es are urrounded by an unstained inte titium. x 200

FIG 10 Dogfi h kidney Fixed 8 µm thick f floating tions Incub tion time 12 min (A) 9 min (B) Counter taining with hematoxyli and eo in (A) Th kidney tub l s re unstained except f ingl cell (rows) N t that thes cell have bas l position and do not reach th tubular lumen I B stained glomerul i en t the uppe left; the taining i not due t trapped rythrocytes x 200

al 1955) Histochemically the tubular cells showed no enzyme activity except for some unidentified cells interspersed between them Fig 10A B These cells always had a basal position and were never found to reach the tubular lumen No attempt was made to study their distribution along the nephron The glomeruli were however stained Fig 10B The function of this enzyme in the glomeruli and the few stained cells of the kidney tubules is not clear at present However the finding that all tubular cells in contact with the urinary fluid lack carbonic anhydrase corresponds with the low activity of the homogenates and with the lack of effect of acetazolamide in vivo on the urinary composition It would also indicate that the ability to alter the urinary pH is associated with the presence of carbonic anhydrase in the kidney tubules since all vertebrates with ability to regulate the urinary pH have tubular enzyme (see above)

The dogfish rectal gland is a NaCl secreting organ and contains considerable amounts of carbonic anhydrase whereas no enzyme is found in the skeletal muscle Table VII This goes along with the present findings that this gland showed clear histochemical staining of the whole epithelium Fig 11 A whereas the muscle fibers were unstained Fig 11 B

Single nerve cells of the central nervous system of the rat were found by Giacobini (1961) to have a very low carbonic anhydrase activity 1/670th of that of erythrocytes The glia cells have 120 times

FIG 11 Dogfi h re t l gland (A) and kel tal muscl (B) Fixed 8 µm thick f e floating sections Incubation time 9 min (A) 12 min (B) Th whol r ctal gland epithelium is stained (A) Th mus le fibers ar unstained (B); arrow point t tained capillary x 300 (A) x 160 (B)

FIG 12 Rat c rvical spinal cord (A) and choroid plexus (B) Fixed 4 µm thick s ctions on Millipo ® filt rs Incubation time 15 min (A) 6 min (B) N te unstained triangularly haped n rve c ll in th center f A It is surrounded by tained tructures po ibly f glial origin B shows stained choroid plexus pithelium upported by un tained stroma 1000 (A) 400 (B)

FIG 13 Erythrocytes insid essel in monkey kidney medulla Fixed 8 µm thick free fl ating tion I cub tion time 2 min x 1000











13

TABLE VII Correlation between biochemical and histochemical tests for carbonic anhydrase activity in various tissues

Tissue	Species	Biochemistry enzyme units/g wet weight: present findings	Biochemistry enzyme units/g wet weight: literature data[b]	Histochemistry[c]
dorsolat. prostate	rat	2670 (n=1)	2400	Fig 9A
ventral		2 (n=1)	28	9B
kidney	dogfish	2 (n=3)	0-12	10A,B
rectal gland		210-216 (n=2)	92 204	11A
muscle		2 (n=1)	0	11B
nerve cells (CNS)	rat		4[d]	12A
choroid plexus epithelium			900[d]	12B
erythrocytes	various mammal	1400-2200	1100-2400	13
parietal cells	rat		7200	(11)
proximal tubules	man		490[e]	(27)
distal			790[e]	(27)
corneal endothelium	rabbit		350 (28)	(28)
epithelium stroma			1 (28)	(28)
lens			195	+ (3)
ciliary processes			23	[g] (3,11)
retina			50	[g] (3,40)
duodenum	rat		77	[g] (11)
bladder mucosa	turtle		24 (54)	+[g] (49)
skin	frog		74[f] (52)	[g] (52)

[a]Each homogenate tested represents one animal

[b]References not found in Maren (1967b) are given with the data

[c]Fig. numbers refer to the present study. Figures in brackets are references to literature data. + staining, – no staining

[d]Calculated from Giacobini (1961) assuming a value of 2400 e.u./g rat erythrocytes

[e]Calculated from Lee and Mattenheimer (1964) assuming a value of 1400 e.u./g human erythrocytes

[f]E.u./g dry weight

[g]Only some cells were stained

and the choroid plexus cells 250 times higher activity than the nerve cells respectively This offered a possibility to compare biochemical and histochemical findings in cells rather than in whole tissues

Rat brain and cervical spinal cord were studied histochemically The staining pattern in unfixed sections could not be interpreted due to badly preserved tissue structure In fixed sections however the large pyramidal cells in the cerebral cortex the Purkinje s cells in the cerebellum and the large motor neurons in the ventral horn of the spinal cord could be identified with certainty These nerve cells showed no enzyme activity Fig 12 A They were surrounded with dis tinctly stained structures which could not be identified but may re present glia In the choroid plexus the surface epithelium was intensely stained whereas the supporting stroma was unstained Fig 12 B

The erythrocytes of all mammalian species tested contain large amounts of carbonic anhydrase Table VII Histochemically the erythrocytes seen in fixed tissues of man monkey rabbit rat and mouse were heavily stained after short incubation times of 1 2 min Fig 13 In unfixed tissues however they generally failed to produce distinct staining In an effort to clarify this discrepancy a droplet of eryth rocytes freshly suspended in saline was deposited on a Millipore[R] filter dried and incubated The staining was diffusely distributed as a precipitate on the filter paper as previously reported also by Bhattacherjee (1971) It is probably due to loss of enzyme from the disrupted unfixed cells during the incubation

Previous studies of various tissues with Hansson s method have also demonstrated that the histochemical findings agree with data on enzyme activity obtained by other methods see Table VII Thus in the cow and rabbit cornea only the endothelial cells are histochemically acti ve which correlates with biochemical data on dissected parts of these corneas (Lönnerholm 1972 1974 Silverman and Gerster 1973) The lens ciliary body and retina show enzyme activity biochemically (see Maren 1967 b) and histochemically (Hansson 1968 Bhattacherjee 1971 Musser and Rosen 1973 a Lönnerholm unpublished observations) in several mammalian species Hansson (1968) demonstrated carbonic anhydrase activi

ty in several actively transporting cells known or suspected to have the enzyme among them parietal cells and duodenal and gall bladder epithelial cells In the turtle urinary bladder mucosa and the frog skin the histochemical demonstration of single active cells corres ponds with the findings of low enzyme activities in homogenates of these tissues (Rosen 1970 Scott et al 1970 Rosen and Friedly 1973)

In summary the present and previous studies show an excellent correlation between histochemical and biochemical findings This is strong evidence in support of the validity of the histochemical method

SUMMARY

The histochemical method of Hansson which is based on the precipita tion of a cobalt phosphate complex was found to be specific for carbonic anhydrase by the following criteria

1) The activity of purified enzyme preparations as measured biochemi cally was found to agree well with their ability to produce staining in histochemical model experiments The human erythrocyte isoenzyme HCA C was clearly more active than the isoenzyme HCA B in both methods The staining was also well related to the catalytic activity of enzy mes modified by introduction of various metal ions at the active site (metal free apo-HCA B Cu^{2+} Co^{2+} and Zn^{2+} HCA B)

2) The carbonic anhydrase inhibitors acetazolamide and ethoxzolamide in micromolar concentrations inhibited the catalyzed staining in ac tive sections They did not influence the uncatalyzed staining which appears after long incubation in inactive sections (heat inactivated or from tissues lacking enzyme) nor did acetazolamide influence the precipitation of phosphate (^{32}P) in inactive sections These findings indicate that the inhibitors do not interact critically with any compo nent of the incubation medium but inhibit the histochemical staining in a specific manner

3) The relative potency of structurally highly different sulfonamides as inhibitors of the histochemical staining reaction was found to correlate well with the inhibitory effect of these drugs as determined kinetically

4) Intravenous injection of physiologically active doses of acetazolamide 20 100 mg/kg to rats inhibited the histochemical staining ith a clear dose response relationship

5) Histochemical demonstration of carbonic anhydrase in various tissues was found to correspond well with biochemical data Both methods show high activity in erythrocytes of several species rat dorsolateral prostate rat choroid plexus epithelium and dogfish rectal gland No or low activity is found in rat ventral prostate nerve cells of rat CNS and dogfish muscle

6) Histochemically the tubular cells of the dogfish kidney showed no enzyme activity except for a few unidentified cells This corresponds with the low activities found in homogenates and with the lack of effect of acetazolamide in vivo on the urinary composition

Th present work was uppo ted by grant 04X 2874 from th Swedi h Medical Re ea ch Council and by grants f om Regn llska donationsfonden and Svenska Sä11 kap t fö Medi insk F rskning

I wish t thank Mr Donna Lentz for kilful technical as i tanc

39 MILLER D M. Total solubilization f erythrocyte membranes by non-ionic detergents Biochem Biophy Res Commun 1970 40 716-722
40 MUSSER G L and S ROSEN Localization f carbonic anhydrase activity in the v rtebrate retina Exp Eye Res 1973a 15 105-119
41 MUSSER G L and S ROSEN Ca bonic anhydrase activity in primate photorec ptors Exp Eye Res 1973b 15 467-470
42 MUSTAKALLIO K.K J RAEKALLIO and E RAEKALLIO The histochemi cal demonstr tion f carbonic anhyd ase An attempt to localize its inhibition by acetazolamide (Diamox®) in rat kidney Ann Med Exp Biol Fenn 1960 38 247-251
43 MUTHER T F A critical evaluation f th histochemical method for carbonic anhydrase J Hi tochem Cytochem 1972 20 319 330
44 NYMAN P O Purification and properties of carbonic anhydras from human erythrocyte Biochim Bi phys Acta 1961 52 1 12
45 PEARSE A G.E Histochemistry theor tical and applied 3rd ed London Churchill Ltd 1968
46 PESETSKY I Carbonic anhyd ase activity in ependymoglial cells of lowe vertebrates Histochemie 1969 19 281 287
47 PHILPOT F J and J St L PHILPOT A modified colorimetric estimation of carboni anhydras Biochem J 1936 30 2191-2194
48 RAZIN S and V BARASH Solubilization f mycopl sma memb anes by the nonionic detergent Triton X 100 FEBS Letters 1969 3 217 220
49 ROSEN S Localization f ca boni anhydras activity in trans porting urinary pith li J Histochem Cytochem 1970 18 668-670
50 ROSEN S Localization f c rboni anhydrase activity in the vertebrate neph on Hi tochemical J 1972a 4 35-48
51 ROSEN S Localization of ca bonic anhydrase in turtle and toad u inary bladder mucosa J Hi tochem Cytochem 1972b 20 696-702
52 ROSEN S and N J FRIEDLEY Carbonic anhydras ctivity in Rana pipiens skin: biochemi al and hi tochemical analysis Histochemi 1973 36 1 4
53 ROSEN S and G L MUSSER Ob ervations on the pecifi ity f neve histochemical methods f r th demonstration f carbonic anhydrase activity J Histochem Cytochem 1972 20 951-954
54 SCOTT W N Y E SHAMOO and W A BRODSKY Carbonic anhydras content f turtl urinary bladd muco al cells Biochim. Biophys Acta 1970 219 248-250
55 SILVERMAN D N and R GERSTER Th detection and localization f carbonic anhydrase in th abbit cornea Exp Eye Res 1973 17 129-136
56 TAYLOR P W R W KING and A S V BURGEN Influence of pH on th kinetics of omplex f rmation between aromatic s lfonamides and human ca boni anhydras Biochemi try 1970a 9 3894 3902
57 TAYLOR P W R W KING and A S V BURGEN Kinetics of omplex formati b tween human carbonic anhyd ases and aromati s lfonamides Biochemistry 1970b 9 2638-2645

58 WALDEYER A. and G HÄUSLER Histochemische Studien über die Carboanhydra e-aktivität de Samenwege und ihr Anhangsdrüs n von Mus ttus Acta biol med germ. 1959 2 568 589
59 WISTRAND P J Comparison by direct measurement of sulfonamide carbonic anhydr se inhibition in whole c lls and in enzyme solutions Drugs Enzymes 1965 4 175-186
60 WISTRAND P J S LINDAHL and T WÄHLSTRAND Human carbonic anhyd ases A kinetic comparison betwe n renal and erythrocyte isoenzymes 1974 Submitt d for publication
61 WISTRAND P J and S N RAO Immunol gic and kinetic properties f arboni anhyd ase from various tissues Biochim Biophys Acta 1968 154 130-144
62 WISTRAND P J J A RAWLS and T H MAREN S lphonamide arboni anhydras inhibito and int a-ocul r p essure in rabbits Acta Pharmacol Toxicol 1960 17 337 355

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum n:o 419

THE VOLUNTARY CONTROL IN HUMAN BREATHING

BY

PEKKA K. HALTTUNEN

HELSINKI 1974

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum n:o 419

THE VOLUNTARY CONTROL IN HUMAN BREATHING

BY

PEKKA K. HALTTUNEN

HELSINKI 1974

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum n:o 419

FROM THE INSTITUTE OF PHYSIOLOGY UNIVERSITY OF HELSINKI,
AND THE DEPARTMENT OF PULMONARY DISEASES
UNIVERSITY CENTRAL HOSPITAL
HELSINKI, FINLAND

THE VOLUNTARY CONTROL IN HUMAN BREATHING

BY
PEKKA K. HALTTUNEN

HELSINKI 1974

Hämeen Kirjapaino Oy
Tampere 1974

PREFACE

This investigation was carried out at the Institute of Physiology University of Helsinki, and at the Department of Pulmonary Diseases University Central Hospital, Helsinki.

I am especially grateful to the Head of the Institute Professor R. M. Bergström, M.D who generously placed the technical resources of the Institute at my disposal. I am also grateful for the constant advice and encouragement, as well as for the critical comments he made during the course of the work.

To my teacher Docent A. A. Viljanen, M.D I am indebted for advice in regard to problems concerning regulation of breathing and also recording respiratory functions. His encouragement and constructive criticism have been of great value to me in the experiments and during the preparation of the manuscript.

The useful advice and valuable remarks of Docent V Virsu, Ph.D have been greatly appreciated.

My sincere thanks are due to all the members of the staff of the Institute of Physiology and especially to Mr R. Blåfield, C.E., for his skilful technical assistance and to Mr J Erosuo B.M. and Mr A. Harjula, B.M., for their continuous interest and valuable assistance during time-consuming experiments. I am also grateful to Mr M. Hyvärinen for his help at the beginning of my work.

Grateful acknowledgement is given to Mrs Leena Manner Cand. Phil. and Mr O Salakari, of the Computing Centre University of Helsinki, for their advice in preparation of all the statistical analyses.

My thanks are also due to Miss Mirja Sisko Lyyra for translating the manuscript and to Mr K. Hart for checking it.

I wish to extend my thanks to Mrs Impi Lieska and Mrs Marjatta Mustala for typing the manuscript and to Miss Hanna Alanen for drawing the figures.

My sincere gratitude is expressed to the courageous experimental subjects and many other persons who offered their help during the course of the investigation.

Helsinki, May 1974

Pekka Halttunen

CONTENTS

1. INTRODUCTION 7
2. REVIEW OF LITERATURE 9
2.1. Voluntary control of skeletal muscles 9
2.2. The neural control of respiratory muscles 11
3. THE RELATIONSHIP BETWEEN THE SUBJECTIVE MAGNITUDE AND THE VOLUME OF INSPIRED AND EXPIRED AIR PRODUCED VOLUNTARILY BY THE RESPIRATORY MUSCLES 13
3.1. Material and method 13
— Subjects 13
— Measurement of respiratory volume 13
— Psychophysical procedures 14
3.2. Results 16
— Category production 16
— Magnitude estimation 17
— Discrimination 19
4. THE ELECTRICAL ACTIVITY OF INTERCOSTAL MUSCLES AND SENSATION MAGNITUDE DURING VOLUNTARY INSPIRATION 21
4.1. Material and method 21
— Subjects 21
— Registration of electrical activity 21
— Psychophysical procedures 22
— Registration of mechanical activity 22
4.2. Results 23
— The relationship between the voluntary sensation and the number of EMG impulses 23
— The relationship between the voluntary sensation and EMG impulse frequency 23
5. THE INFLUENCE OF CARBON DIOXIDE STIMULATION ON THE RELATIONSHIP BETWEEN SENSATION MAGNITUDE AND VOLUME PRODUCED BY THE RESPIRATORY MUSCLES 25
5.1. Material and method 25
5.2. Results 26
— Category production 26
— Magnitude estimation 27
— The comparison between normal conditions and carbon dioxide stimulation 29
6. DISCUSSION 31
7. SUMMARY 35
8. REFERENCES 36
9. APPENDIX 39

1. INTRODUCTION

Breathing is controlled both by autonomic and voluntary centers. A close relationship has been found earlier between the electrical and mechanical activity of the human respiratory muscles (VILJANEN 1967). Furthermore, in animal experiments, VILJANEN (1970, 1972) has shown a close linear relationship between the phrenic multifiber activity and the corresponding electromyogram impulse activity registered from the diaphragm. Likewise in preliminary investigations indications of a relationship between the subjective sensation and the electrical activity of the respiratory muscles (VILJANEN 1970, 1972) and the mechanical activity of the respiratory muscles (BERGSTRÖM, HALTTUNEN & VILJANEN 1972) have been found.

Since the available information does not adequately clarify the physiological mechanism underlying the voluntary control of breathing, it is appropriate to investigate this matter by a sensory-physiological measuring method, which is considered to highlight the gross activity of the higher centers of the brain. This calls for an investigation in which relationships between the subjective sensation and mechanical activity of the respiratory muscles and subjective sensation and electrical activity of the respiratory muscles are studied as well as the increased effect of the carbon dioxide stimulation on the said relationship. The latter partial investigation can be considered to clarify further the role autonomic control plays in the voluntary control.

In the present study the following problems are studied.

The relationship between the subjective sensation and the olumes produced by the respiratory muscles when they are controlled voluntarily

The relationship between the subjective sensation and the lectrical activity produced by the intercostal muscles during voluntary inspiration

The effect of an increased carbon dioxide stimulation on the relationship between subjective sensation and respiratory volumes.

2 REVIEW OF LITERATURE

2.1. *Voluntary control of skeletal muscles*

In 1930 in experiments on the skeletal muscles REENPÄÄ (RENQVIST 1930) found a distinct relationship between the physical force of muscle (force force impulse) and the corresponding sensation quantity Later a linear relationship was demonstrated between the force produced by the skeletal muscles and the corresponding electromyogram impulse frequency (JALAVISTO et al. 1939) A similar relationship has also been found between the number (Periodenzahl) of impulses registered from the voluntary muscle and the corresponding physical force impulse (BERGSTRÖM 1959) Furthermore BERGSTRÖM (1957 1959) in experiments on the human skeletal muscles, has compared sensation quantity to electrical activity of the muscle on one hand, and to the mechanical activity on the other hand, and found that both electrical and mechanical parameters demonstrate well the subjective contents which control muscular function, this sensation being best represented by the number of EMG impulses. It is to be assumed that the subjective contents represent the macro state of the organic process of the brain (BERGSTRÖM 1973)

STEVENS (1959) has extensively investigated with psychophysical procedures the correlation between the magnitude of sensation and the corresponding stimulus quantity with various senses. He separates three classes of sensory scales in psychophysics

1 Discriminability scales. These are constructed in the tradition of Fechner whose century-old dogma is that the sensation intensity grows as the logarithm of stimulus intensity grows (The Fechner law)

According to this

$$y = b \log x$$

where y is the magnitude of sensation, x the intensity of stimulus and b is a constant. This function produces a straight line in a semilog coordinate system y plotted against log x.

Some measure of jnd, variability confusion, or resolving power is employed as a unit, and a scale is constructed by counting off such units.

2. Category scales (partition scales) These are constructed by one or another variation of the procedure that PLATEAU invented when he required observers to divide a segment of a continuum into equal appearing intervals. According to STEVENS (1959) the results in a category scale do not follow any simple function

3. Magnitude scale Here the observer simply estimates the apparent strength or intensity of his subjective impressions relative to a standard set either by himself or by the experimenter He found that human subjects are able to make consistent estimations of the subjective quantities. In addition various senses followed the same function the power law when subjective magnitude is expressed as a function of the objective magnitude This function forms a straight line in log log coordinate system

$$y = ax^{b} \rightarrow \log y = \log a + b \log x$$

Later this procedure has also been used for investigating the relationships between sensation quantity and the corresponding physical force in the skeletal muscles. A distinct relationship yielding the power function with an exponent value of 1 7 (STEVENS & MACK 1959) was found between the force of handgrip and the experience of force exerted. EISLER (1962) studied, using the same method, the relationship between the muscular force of the leg and the corresponding sensation quantity and obtained similar results

In the experiments on human respiratory muscles, also relationships between the sensation quantity in breathing and the number of EMG impulses from the respiratory muscles (VILJANEN 1970) as well as between sensation quantity and the EMG impulse frequency have been observed (VILJANEN 1972). A clear relationship has also been verified in preliminary experiments between subjective sensation and the volume produced by the respiratory muscles (BERGSTRÖM HALTTUNEN & VILJANEN 1972).

2.2. The neural control of respiratory muscles

In earlier studies on the neural control in human breathing several relationships have been verified between the electrical activity and the mechanical activity of the respiratory muscles *during voluntary inspiration* (VILJANEN 1967)

1. The total EMG impulse number bears a linear relationship during the course of inspiration to the first time integral of the inspiratory muscular work, the so-called physical action.
2. The EMG impulse frequency registered at the end of inspiration bears a linear relationship to the inspiratory muscular work.
3. Four seconds after the end of inspiration with the subject holding his breath the EMG impulse frequency was in direct proportion to the muscular force
4. During one and the same inspiration the EMG impulse number is directly proportional to the pressure impulse. According to this the EMG impulse frequency during inspiration *is directly proportional to the inspiratory muscle force.*

BERGSTRÖM and KERTTULA (1961) have found in animal experiments that the linear relationship between the number of EMG impulses registered from the intercostal muscles and the corresponding mechanical activity remained the same during carbon dioxide stimulation. Likewise VILJANEN (1967) found in his experiments on man that the linear correlation between the number of EMG impulses and physical action of inspiration remains the same during CO_2 stimulation but the regression

coefficient changes so that the equivalent physical action can be produced by a smaller number of impulses.

The EMG activity registered from the diaphragm has been found to be directly proportional to the corresponding activitv of the intercostal muscles during inspiration (VILJANEN & HALTTUNEN 1967 VILJANEN 1972) In animal experiments a nearly linear relation has been found between unit activity of the phrenic nerve and the electrical activity of the diaphragm (DITTLER & GARTEN 1912, GASSER & NEWCOMER 1921) The same linearity has also been demonstrated between the multi fiber activity of the phrenic nerve and the electrical activity of the diaphragm (VILJANEN 1970 1972) Furthermore, in animal experiments a close relationship has been observed between the afferent neural drive and the efferent electrical activity of the respiratory centers (VILJANEN 1970 1972)

Even though the diaphragm and the intercostal muscles are controlled by the same regulatory system they have been found to have partly at least, a different regulation. Their interactivity can be voluntarily controlled (VILJANEN & POP PIUS 1968 VILJANEN 1970 1972) Further in experiments on the cat, as the level of anaesthesia deepens, the electrical activi ty of the intercostal muscles decreases more than that of the diaphragm (VILJANEN et al 1968 VILJANEN 1970) The latter fact would support the notion that the diaphragm is regulated by a considerably more primitive and more powerful autonomic center than the intercostal muscles On the other hand the num ber of muscle spindles in the intercostals has been established to be greater than that of the diaphragm (DOGIEL 1902, GREGOR 1904 HINSLEY et al. 1939 WINCLER & DELALOYE 1957) This may suggest that the intercostal muscles can give more afferent input to respiratory centers, and this feedback is more intensively controlled also by the higher respiratory centers than the dia phragm while the latter may be more primitive and intensively controlled by the autonomic centers. It has been established earlier that the muscle spindles in the intercostal muscles and their reflexes play an important part in the regulation of respi ration (CAMPBELL & HOWELL 1962, VON EULER 1966 SEARS & NEWSOM DAVIS 1968)

3. THE RELATIONSHIP BETWEEN THE SUBJECTIVE MAGNITUDE AND THE VOLUME OF INSPIRED AND EXPIRED AIR PRODUCED VOLUNTAR ILY BY THE RESPIRATORY MUSCLES

3 1 Material and method

Subjects

The experimental subjects were 12 young healthy students, mostly graduate students of medicine and physical education 8 males and 4 females, aged from 19 to 31 The experiments were carried out during autumn 1971 and spring 1972.

Measurement of respiratory volume

The experiments were carried out in a quiet room. The temper ature was 20°C. During the registration the subjects were in a supine position. During the experiment all tight clothing had been either taken off or at least opened in order to give space for free movements of the thorax. Before the beginning of the experiment the subject rested for about 10 minutes.

A single session lasted from 45 to 90 minutes. To avoid possi ble hypocapnia in the blood, 5—10 quiet breathings separated the relevant breathings, and there were 1—2 pauses 5—10 min utes in duration. A mouth gag was placed into the subject s mouth and breathing was performed through it. In order to exclude breathing through the nose a nose clip was employed

as well. In order to avoid condensation of humidity the inspiratory air was warmed by electrical resistance. During the experiments 1872 inspirations and 2101 expirations were registered.

Respiratory volume which represents the mechanical activity of the respiratory muscles was recorded with a Fleischpneumotachographhead. An electric integrator transformed the flow into volume. The results were visualized on a four-channel Mingograph plotter from which the respiratory volumes were manually measured. After each experiment the volume was calibrated with a piston pump of known volume. The recording system is seen in Figure 1.

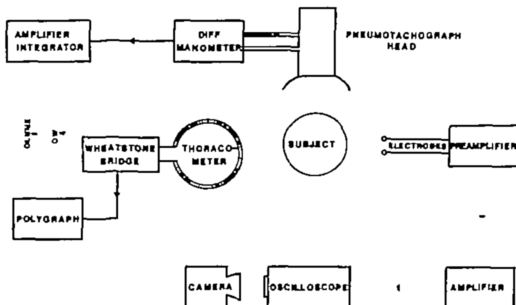


Fig. 1. The recording system.

Psychophysical procedures

Three separate psychophysical classes of sensory scales were construed (See STEVENS 1959)

1. Category scale
2. Magnitude scale
3. Discriminability scale

To obtain category scale category production was used. In

inspiratory experiments numbers were named by the experimenter *in an irregular order from one to ten* where zero represents the functional residual capacity (FRC) and ten the state of maximal inspiration. The subject was instructed to inspire volumes of air whose subjective magnitudes were equally spaced among the ten categories presented by the experimenter Expiration was studied in a similar manner The subjects always started from the FRC-state and then inspired maximally which was called the total lung capacity (TLC) The subject then expired in random order volumes between one and *ten, with ten representing the maximal expiration.*

Magnitude estimation was used to obtain a magnitude scale. The subject inspired from the FRC-state until the experimenter signaled that a sufficient volume had been achieved and then the subject estimated its apparent magnitude using numbers that seemed to him proportional to the magnitude

The magnitude estimation of expiration was studied in the same manner so that the subject always started from the FRC-state and then inspired maximally holding his breath, the TLC state Then the subject expired slowly until the experimenter signalled him to stop. Then the subject estimated the expired volume *using numbers that seemed to him proportional* to the magnitude.

In order to obtain discriminability scale a *just noticeable* difference was produced. In inspiration the subject inspired volumes, starting from the FRC-state that seemed to him just greater than the previous volume After each volume the subject made five to ten quiet inspirations and then returned to the FRC-level inspiring a subjectively just greater volume than the previous one In this way the subject continued up to the maximal volume.

The expiration was studied in the same manner except that the subject started from the TLC-state and expired just noticeably greater volumes.

3 2 Results

Category production

As category production does not yield any simple function in logarithmic coordinates (STEVENS 1959) data obtained with this method are presented in linear coordinates in the present study

Figure 2 shows the function obtained in the inspiration and expiration experiments performed on 12 subjects The regression coefficient of the inspiratory function was 2.51 and the Pearson correlation coefficient was 0 77 ($p < 0\ 001$ $df = 9$) The

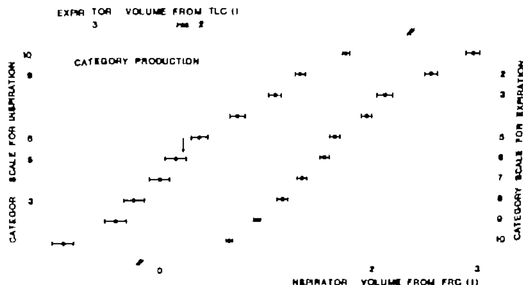


Fig 2 The subjective sensation on the category scale as a function of inspiratory (o) and expiratory (●) volume in a linear coordinate system. The points with ± 1 standard error of the mean are means of 10 subjects. The coefficient of linear regression in inspiration was 2.51 and in expiration 2 11 Notice the FRC level in expiration.

correlation coefficient indicates the interindividual consistency of estimation. The value of *p* was obtained from Fisher's statistical tables. The regression coefficient of the expiration function was 2.11 and the Pearson correlation coefficient 0.75 ($p <$

0 001 $df = 9$) On the ordinate the sensation quantity varies between one and ten. The respiratory volume is on the abscissa. The figure shows the means and the standard errors of the means (interindividual variation $df = 9$ for inspiration and 9 for expiration) Figure 3 shows the results for each subject and the intraindividual variability of the means.

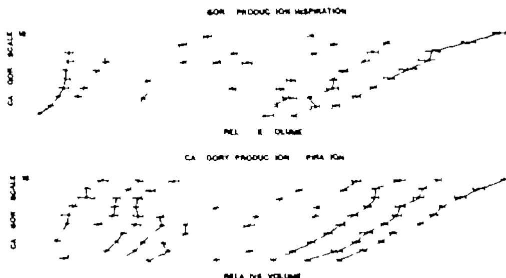


Fig 3. The results of individual subjects in a linear coordinate system The individual regression coefficients and Pearson correlation coefficients are seen in the Appendix. The origin of the abscissa scale is arbitrary but the distance between each scale mark is one liter

Magnitude estimation

Following STEVENS (1959) the results are presented in log log coordinates.

The results are shown in Figure 4 and appear to follow the power law The magnitude is scaled from one to ten. The coeffi cient of log log regression (the value of the power law exponent) was 1 00 in inspiration and the Pearson correlation coeffi cient 0.57 ($p < 0.05$ $df = 9$) The corresponding regression coefficient of the expiration was found to be 1 03 and the Pear

son correlation coefficient 0 68 ($p < 0\ 01$ $df = 9$) The standard deviation of the individual regression coefficients was 0.417 ($n = 10$) in inspiration and 0 279 ($n = 10$) in expiration Figure 5 shows the results for each subject.

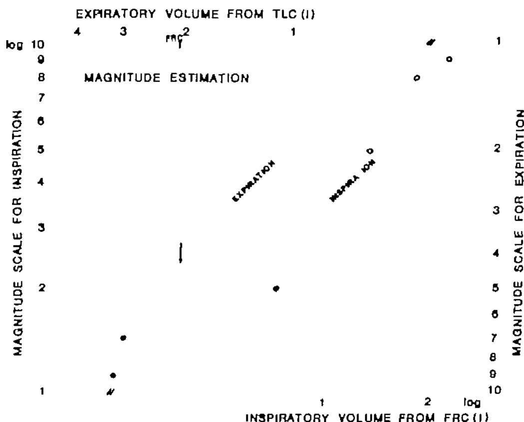


Fig 4 The subjective sensation as a function of inspiratory and expiratory volume in log-log coordinates The magnitude scale is scaled from one to ten The points are means of the results of 10 subjects.

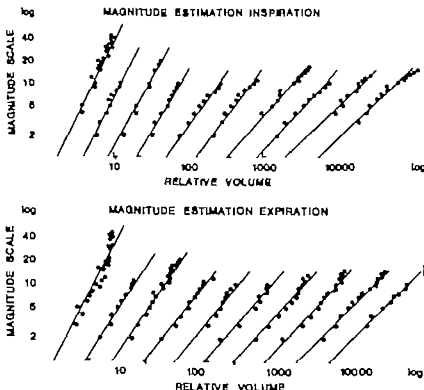


Fig 5 The individual results in a log log coordinate system. The lines have been displaced by integral factors to facilitate presentation. The individual regression coefficients and Pearson correlation coefficients are seen in the Appendix.

Discrimination

Following STEVENS (1959) the results are presented in a semilog coordinate system. The results are shown in *Figure 6*. Discrimination was scaled from one to ten. The results do not follow the Fechner law as can be seen from the curvature of the resulting functions. In inspiration the coefficient of the semilog regression was found to be 3 02 and the Pearson correlation coefficient 0 37 ($p < 0\ 05$ $df = 4$) In expiration the corresponding values were 2.93 and 0.70 ($p < 0\ 001$ $df = 4$) Figure 7 shows the results for each subject.

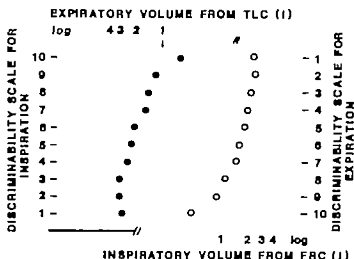


Fig 6. The subjective sensation on the discriminability scale as a function of inspiratory (o) and expiratory (●) volume in a semilog coordinate system. Discrimination is scaled from one to ten. The points are means of 10 subjects. The regression coefficient in inspiration was 3.02 and in expiration 2.93

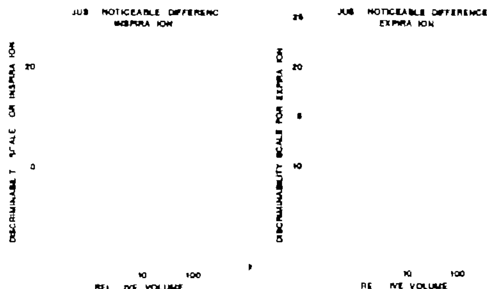


Fig 7. The individual results in semilog coordinate system. The subjects have been displaced by integral factors to facilitate presentation. The individual regression coefficients and Pearson correlations coefficients are seen in the Appendix

4. THE ELECTRICAL ACTIVITY OF HUMAN INTER COSTAL MUSCLES AND SENSATION MAGNITUDE DURING VOLUNTARY INSPIRATION

4.1 Material and method

Subjects

The experimental subjects were 14 young, healthy male stu dents of medicine and physical education aged from 20 to 24 Since the EMG registration was derived from surface elec trodes subjects with a thinck subcutis were excluded. During the experiments 665 inspirations were registered.

Registration of electrical activity

The electrical activity of the intercostal muscles was regis- tered electromyographically The recordings were carried out in a Faraday cage under quiet laboratory conditions. The electro- myograms were registered with bipolar surface electrodes (VIL JANEN 1967) The electrode distance was 25 mm and the di ameter of an electrode 7 mm. The ordinary electrodes were pieces of felt, clamped on plastic and fixed with Tensoplast to the skin, which had been cleaned with ether Before the exper iments, the felt was moistened with electrode fluid

It can be assumed that an EMG activity sample obtained by surface electrodes represents the total electrical activity of the intercostal muscle (LIPPOLD 1952, BERGSTROM & KERTTULA

1961) The following measurements were calculated visually from the film. 1 The total number of EMG impulses (*n* num ber) during inspiration. 2. The EMG impulse frequency during the last 1/3 second of inspiration.

The EMG impulses were counted by two persons who did not know each others results, and a third checked the counting by arbitrarily selected samples. All impulses which were distin guishable from the baseline were counted as EMG impulses The method was the same as that used by BERGSTRÖM (1958) and by VILJANEN (1967)

Psychophysical procedures

The subjective-multiple method that belongs to the category group was used in the experiments.

Before the beginning of the experiment the smallest volume at which EMG could be registered was sought. When it was found it was determined as the first volume on the subjective scale. Generally it varied between 0 5 and 0 75 liters After this the subject was instructed to draw given subjective multiples of the base value. Each subjective multiple was registered ten times, the mean number of multiples being six. The number of multiples varied from 4 to 9 and the maximum corresponded to the maximal inspiration.

Registration of mechanical activity

Recording of the inspiratory volume and flow was performed on a Fleischpneumotachograph head, by means of an Elema Schönander differential manometer electrical integrator and a four-channel polygraph Mingograph 42. After each experiment the volume was calibrated with a piston pump of known vol ume

4.2. Results

The relationship between the voluntry sensation and the number of EMG impulses

As in section 3 the results are shown in linear coördinates Figure 8 shows the function obtained during inspiration experiments on all subjects The subjective sensation is scaled on the ordinate one to ten and on the abscissa the total number of EMG impulses registered from intercostal muscles during inspiration. The figure shows the means and the standard errors of the means. The regression coefficient was found to be 0 01 and the Pearson correlation coefficient 0 61 ($p < 0 01$ $df = 13$)

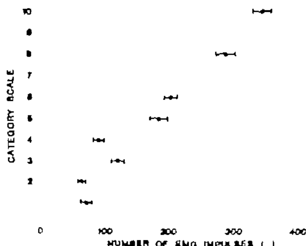


Fig 8. The subjective sensation as a function of the total number of electromyogram impulses during inspiration in a linear coordinate system. The points with ± 1 standard error of the mean are means of 14 subjects. The sensation is scaled from one to ten The coefficient of linear regression was 0.01.

The relationship between voluntary sensation and the EMG impulse frequency

Figure 9 shows the relationship between the subjective sensation and the EMG frequency The sensation is scaled from one

to ten. The regression coefficient was found to be 0 03 and the Pearson correlation coefficient 0 49 ($p <$ 0 05 $df = 13$)

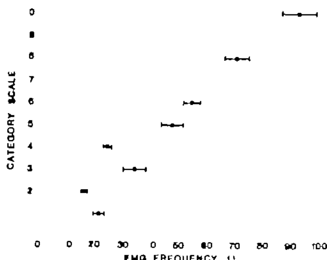


Fig 9. The subjective sensation as a function of electromyogram impulse frequency during inspiration in a linear coordinate system The points with ± 1 standard error of the mean are means of 14 subjects The coefficients of linear regression was 0 03.

5 THE INFLUENCE OF CARBON DIOXIDE STIMULATION ON THE RELATIONSHIP BETWEEN SENSATION MAGNITUDE AND VOLUME PRODUCED BY THE RESPIRATORY MUSCLES

5 1 Material and method

The experimental subjects were 8 healthy young students aged from 20 to 29 Six of the subjects had previously participated in physiological investigations performed by means of psychophysical methods, and two of them were novices

The experimental procedure was the same as in the preceding study The subjects breathed in through a flap valve from a plastic bag containing 2000 liters 5.0 ± 0 5 % CO_2 air and expired through another valve into the atmospheric air

The following psychophysical methods were used in the experiments.

1 Category production
2. Magnitude estimation

The cource of the experiments corresponded with that of similar experiments performed in normal conditions. During the experiments 1576 inspirations and 1443 expirations were registered.

Category production

During the CO_2 stimulation the regression coefficient of the inspiration function was found to be 2.65 in a linear coordinate system and the Pearson correlation coefficient 0.83 ($p < 0.001$ $df = 7$) The regression coefficient of the expiration function was found to be 2.01 and the Pearson correlation coefficient 0.84 ($p < 0.001$ $df = 6$) The results are shown in Figure 10 Figure 11 shows the results for each subject.

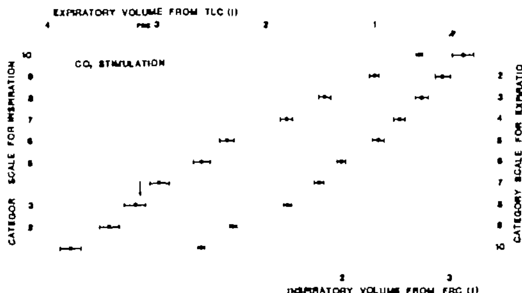


Fig 10 The subjective sensation on the category scale as a function of inspiratory (o) and expiratory (●) volume in a linear coordinate system during CO_2-stimulation The points with ± 1 standard error of the mean are means of 8 subjects. The coefficient of linear regression was in inspiration 2 65 and in expiration 2 01. Notice the FRC level in expiration.

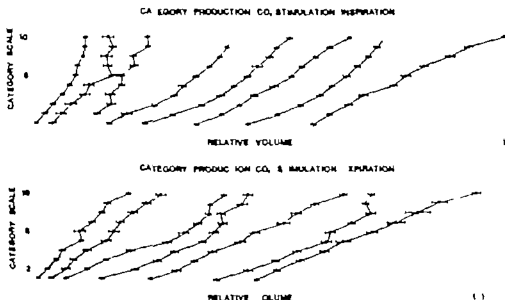


Fig 11. The individual results in a linear coordinate system during CO_2-stimulation. The individual Pearson correlation coefficients and regression coefficients are seen in the Appendix.

Magnitude estimation

The exponent of the power function (coefficient of the log log linear regression) obtained in the inspiration tests was 1 19 and the Pearson correlation coefficient 0 90 ($p < 0\,001$ $df = 7$) In expiration tests the regression coefficient was 0.99 and the Pear son correlation coefficient 0 93 ($p < 0\,001$ $df = 7$) In Figure 12

the results are seen in a log log coordinate system. The magnitude is scaled from one to ten. The standard deviation of the individual regression coefficient was in inspiration 0.307 (n = 8) and in expiration 0 128 (n = 8) In Figure 13 the individual results are shown.

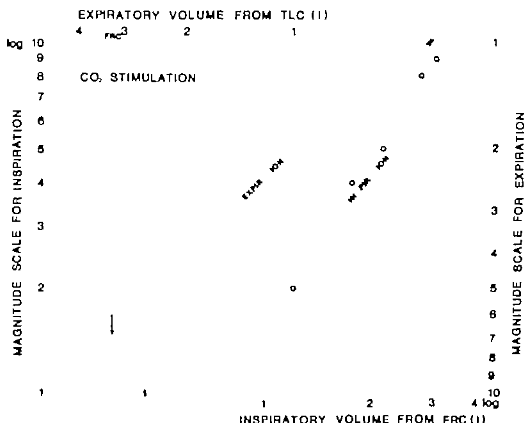


Fig 12. The subjective magnitude as a function of inspiratory and expiratory volume in *log log coordinates during CO_2-stimulation. The sensation* is scaled from one to ten The points are means of the results of 8 subjects

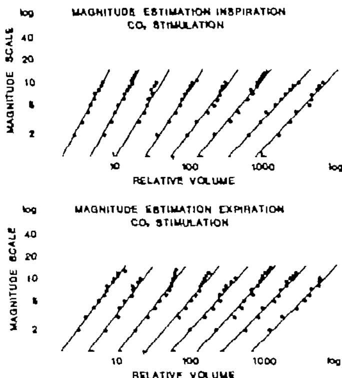


Fig 13. The individual results in a log-log coordinate system during CO_2-stimulation. The lines have been displaced by integral factors to facilitate the presentation. The individual regression coefficients and Pearson correlation coefficients are seen in the Appendix.

The comparison between normal conditions and CO_2 stimulation

On comparing the results obtained with the magnitude meth od both under normal conditions and during CO_2 stimulation it was found that the regression coefficient in inspiration du ring CO_2 stimulation became higher ($t = 5.575$ $df = 1581$ $p <$

0 001) and the cutting point of axis y lower (t = 23 970 df = 1591 $p < 0\ 001$) than under normal conditions. The differences are seen in Figure 14 It can be seen from the figure that a larg er volume was inspired by the same subjective magnitude dur ing CO_2 stimulation.

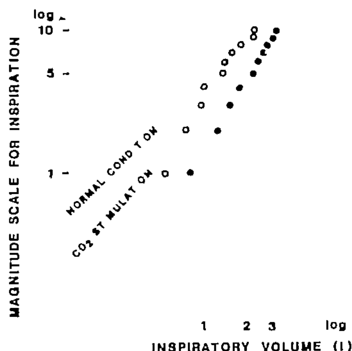


Fig 14. The effect of CO_2 stimulation on the relationship between the voluntary *sensation* and the inspiratory volume. The points are means of the results of 18 subjects

6 DISCUSSION

In attempts to clarify the voluntary control of the human respiratory muscles on a higher central level the greatest diffi culty is measuring these control functions directly When a psychophysical procedure is chosen as a method, the subjective sensation produced by the subjects respiratory muscle move- ments can be used as a yardstick of the higher control which can be considered to represent the voluntary drive to the higher respiratory centers well. Earlier the psychophysical method had been found reliable in experiments made to explore the volun tary control of the human skeletal muscles (STEVENS & MACK 1959 EISLER 1962, BERGSTRÖM, HALTTUNEN & VILJANEN 1972)

In the present study scales of sensation were constructed by using the methods of just noticeable difference category pro duction and magnitude estimation.

Problem 1 Relationship between sensation and respiratory volume

In the present study a consistent relationship between the subjective sensation and the corresponding volume produced by the respiratory muscles was found. This followed the power law with the magnitude method.

In comparing the different methods used in the present study it can be found that the best correlated set of inspiratory and expiratory data was found with the category method. A clear inconvenience in carrying out the discriminability experiments was the fact that the experiments tended to become too long and obvious fatigue was detectable. Furthermore the scales formed by different subjects were quite different and thus less suitable for comparison. Perhaps for that reason, the overall function obtained did not follow the Fechner law

The fact that the category method in these experiments gave the best correlated results can be explained by the fact that in sensory physiological experiments in general the limits of the studied area are unknown to the subject but the respiratory movements present a clearly limited occurrence to the subject, whereby he knows the beginning and the termination points of the action. Under these circumstances the category scale may suit better for this kind of limited range in particular since it is known that the respiratory movements as such are a consid erably more complicated process than normal muscular work, and therefore it is easier for the subject to operate by means of a given scale

In comparing the regression coefficients obtained in inspi ration and expiration it is found that larger coefficients were obtained in inspiration than in expiration exept with the magni tude method. This may suggest that inspiratory and expiratory muscles differ from each other in the amount of higher control, though both are also autonomically controlled Further it can be noticed that the Pearson correlation coefficient in expiratory experiments was higher than in the inspiratory experiments (see Table 1) in other words, the voluntary control of the expir atory muscles would be more accurate (compare vocalization)

In the present experiments the exponent for inspiration was 1 00 and for expiration 1 03 obtained with magnitude estima tion It has been observed earlier that handgrip gives an exponent of 1 7 (STEVENS & MACK 1959) and the muscular force of the leg gave the same (EISLER 1962) It is interesting that the exponent values obtained are so similar in spite of the fact that the physical correlate in the present experiments is not force but a combination of force and work.

Problem 2. Relationship between sensation and electrical activity

In the second part of the present study a clear relationship between subjective sensation and the electrical activity registered from the intercostal muscles during inspiration was also found in the linear coordinate system. In previous experiments on human respiratory muscles a clear relationship was found between the subjective sensation and the total number of EMG impulses registered from respiratory muscles, as well as between sensation and the EMG frequency (VILJANEN 1970 1972) in the present study which used a larger material, it was found that sensation has a higher correlation coefficient to the total number of EMG impulses than to their frequency (see Table 1) BERGSTRÖM (1957 1958) arrived at the same results in his experiments on the skeletal muscles.

Problem 3 CO_2 stimulation

In the third part of the present study the aim was to investigate how the increased CO_2 stimulation would affect the relationship between subjective sensation and the volume produced by the respiratory muscles. It was found that with both methods a higher correlation coefficient was achieved with CO_2 than during normal conditions (see Table 1) Also the inspiratory regression coefficient was found to be higher and the cutting point of axis y lower than during normal conditions. This means that a greater volume was achieved with the same sensation during the CO_2 stimulation.

It has been observed earlier that the same respiratory muscle force is achieved with a smaller number of EMG impulses during CO_2 breathing (VILJANEN 1967) Thus, this kind of CO_2 effect, in which a greater volume equals the same sensation quantity may be closest to a direct involuntary autonomic effect. The results also agree with the observations made in experiments on voluntary muscles (BERGSTRÖM 1957 1958) and

sensory physiological experiments (JARVILEHTO 1973) where sensation has been found to follow the electrical activity better than the mechanical activity Furthermore it has been verified in earlier experiments that the final state of inspiration is determined and after starting the inspiration the impulse pattern no longer changes (VILJANEN 1967 1970)

It may be concluded that the voluntary action controls respiratory functions in the same way as it does the other voluntary muscles The vegetative centers control the respiratory muscles to get an adequate interchange of gases which in turn does not function as a control of the subjective will.

Voluntary control may regulate respiratory movements only as far as these are in the same position as ordinary motor movements, like among other things speech singing eating, diving etc.

The results obtained may be of interest in investigating the interaction between conscious central and autonomic functions.

7 SUMMARY

1. In the present study comparisons were made by means of a psychophysical method between the subjective sensation and respiratory volumes produced by the respiratory muscles. Further the relationship between sensation and EMG activity registered from the intercostal muscles were studied during inspiration. The effect of CO_2 stimulation on the relationship between sensation and volume was also observed

2. The subjective sensation followed the power law with the method of magnitude estimation.

3. The sensation was found to have a higher correlation to total number of EMG impulses than to EMG frequency

4. During CO_2 stimulation a greater respiratory volume equalled the same sensation magnitude

8 REFERENCES

BERGSTROM R M, Die Periodenzahl des Elektromyogramms als Begriffsabbild des Erlebnisses einer voluntären Muskelaktion. Acta physiol. scand. 1957 42 145

— — Über die Anzahl der motorischen Impulse (die Periodenzahl) des Elektromyogramms als quantitativer Ausdrück der voluntären, centralen Kontrolle der Muskelation. Acta physiol. scand. 1958. 43. 349—358.

— — Quantitative aspects of neural macro states. Cybernetic Medicine 1972. 1 9—17

— — P K HALTTUNEN and A A VILJANEN The voluntary regulation of breathing in man. Acta physiol. scand. 1972 84, 428—429.

— — and Y KERTTULA, On the neural control of breathing as studied by electromyography of intercostal muscles of the rat. Ann. Acad. Sci. Fenn 1961. A5. 79. 1—12.

CAMPBELL, E J M and J B L HOWELL, Proprioceptive control of breathing In. De Reuck, A. V S., O'Conner Maeve (Eds.) Ciba Foundation Symposium on Pulmonary Structure and Function. 29—45, J & A. Churchill London 1962.

DITTLER, R Und S GARTEN Die zeitliche Folge der Aktionströme in Phrenicus und Zwerchfell bei der natürlichen Innervation. Z. Bio 1912 58 420—450.

DOGIEL, A S Die Nervenendigungen im Bauchfell, in den Sehnen, den Muskelspindeln und dem Centrum tendineum des Diaphragmas beim Menschen und bei Saugethieren. Arch. Mikroskop Anat. Entwicklungsmech 1902 59 1—31.

EISLER, H Subjective scales of force for a large muscle group. J Exp. Psychol 1962 64 253—257

EULER, C v The control of respiratory movements. In Howell, J B. L., E J M Campbell (Eds) Blackwell Scientific Publications on Breathlessness 19—32 Oxford, England 1966.

FISHER, G H The new form statistical tables University of London Press Ltd. London 1965

GASSER, H. S and H S NEWCOMER, Action currents in the phrenic nerve Amer J Physiol 1921 57 1—26.

GREGOR, A., Ueber die Vertheilung der Muskelspindeln in der Muskulatur des menschlichen Fötos. Arch. Anat. Physiol. Anat. Abt. 1904. 112—196.
HALD A., Statistical theory with engineering applications. London 1962.
HINSLEY J C., K. HARE and R. A. PHILLIPS Sensory components of the phrenic nerve of the cat. Proc. Soc. Extl. Biol. Med. 1939. 41. 411—414
JALAVISTO E., L. KERÄNEN und J SEPPÄLÄ Über die Nachwirkung der statischen Beanspruchung des Muskels. Skand. Arch. Physiol. 1939. 82. 1—28.
JÄRVILEHTO, T Neural coding in the temperature sense. Human reactions to temperature changes as compared with activity in single peripheral cold fibers in the cat. Ann. Acad. Sci. fenn. 1973. B 184. 1—71.
LIPPOLD O C. J The relation between integrated action potentials in a human muscle and its isometric tension. J Physiol. (Lond.) 1952. 117 492—499.
RENQVIST Y Über Spannungsempfindung, Kraftbegriff und Bedeutung der Zeit bei dem Empfindungs-Reizverhältnis. Skand. Arch. f. Physiol. 1930. 59. 53—66.
SEARS T A. and J NEWSOM DAVIS, The control of respiratory muscles during voluntary breathing. Ann. N Y Acad. Sci. 1968 155. 183—190.
STEVENS, J C. and J D MACK, Scales of apparent force. J Exp. Psychol. 1959. 58. 405—413.
STEVENS, S. S., The psychophysics of sensory function. In: Rosenblith, W A. (Ed.) Sensory Communication. Wiley New York 1959.
VILJANEN A. A., The relation between the electrical and mechanical activity of human intercostal muscles during voluntary inspiration. Acta physiol. scand. 1967 suppl. 296. 1—61.
— — The effect of CO_2-stimulation on the relationship between the electrical and mechanical activity in human intercostal muscles. Scand J clin. Lab. Invest. 1967 19 suppl. 95. 65.
— — Pattern of motor activity in breathing in response to varying sensory inputs. In: Porter R. (Ed.) C ba Foundation Symposium on Breathing. Hering Breuer Centenary Symposium. 163—193. J & A. Churchill. London 1970.
— — Co-ordination of neuromuscular efferent system in breathing. Royal College of Surgeons of England, 1972, in print.
— — and P K. HALTTUNEN Comparison of electromyogram activities of the diaphragm and the intercostal muscle in man during voluntary inspiration. Acta neurol. scand. 1967 43. suppl. 31, 191.
— — and H. POPPIUS, The voluntary regulation of contraction of diaphragm and intercostal muscles in man during inspiration. Scand. J clin. Lab. Invest. 1966. suppl. 101. 63—64.
— — Y SALORINNE, L. A. LAITINEN and M. PALOHEIMO The effect of ether anesthesia on the electrical activity of the diaphragm and intercostal muscles during inspiration in the cat. Scand. J clin. Lab. Invest. 1966. 21. suppl. 101. 64.
WINCLER, G et E. DELALOYE, A propos de la présence des fuseaux neuromusculaires dans le diaphragme humain. Acta Anat. 1957 29. 114—116.

APPENDIX

TABLE 1 Numerical values of linear regression coefficients and Pearson correlation coefficients in different experimental groups and with different transformations of the coordinates.

	Category production	Magnitude estimation	Discrimination	Number of EMG impulses	Frequency	Category production	Magnitude estimation
	normal condition					CO^3 stimulation	
Inspiration							
$y = bx + a$	$y = 2{,}31x + 1{,}35$			$y = 0{,}01x + 3{,}45$	$y = 0{,}03x + 4{,}07$	$y = 2{,}65x + 0{,}85$	
Pearson correlation coefficients (lin/lin)	0,77			0,61	0,49	0,83	
$y = b\log x + a$			$y = 3{,}02\log x + 4{,}85$				
Pearson correlation coefficients (lin/log)			0,37				
$\log y = b\log x + \log a$		$\log y = 1{,}00\log x + 1{,}33$					$\log y = 1{,}19\log x + 0{,}73$
Pearson correlation coefficients (log/log)		0,57					0,90
Expiration							
	$y = 2{,}11x + 1{,}18$					$y = 2{,}01x + 0{,}81$	
rrelation (lin/lin)	0,75					0,84	
			$y = 2{,}93\log x + 3{,}17$				
correlation (lin/log)			0,70				
		$\log y = 1{,}00\log x + 0{,}74$					$\log y = 0{,}99\log x + 0{,}79$
		[illegible]					0,91

TABLE 2 Numerical values of individual regression coefficients and Pearson correlation coefficients in different experimental groups

CATEGORY PRODUCTION

Inspiration

subject and experiment code	scale	number of inspirations and expirations	regression coefficient in linear coordinates	correlation coefficient in linear coordinates	regression coefficient in log log coordinates	correlation coefficient in log-log coordinates
43	1—10	49	3,18	0,79	1,25	0,79
44	1—10	50	2,64	0,97	1,27	0 96
46	1—10	100	2,11	0,94	1,07	0,94
52	1—10	100	3,79	0,96	1,42	0 96
53	1—10	55	3,83	0,97	1,34	0,96
54	1—10	50	3,62	0,96	1,22	0 95
55	1—10	50	3,00	0,72	0,86	0,75
61	1—10	80	5,19	0,85	1,87	0,86
62	1—10	100	6,44	0,94	1,46	0,95
63	1—10	100	5,46	0,93	1,58	0,93

Expiration

subject and experiment code	scale	number of inspirations and expirations	regression coefficient in linear coordinates	correlation coefficient in linear coordinates	regression coefficient in log log coordinates	correlation coefficient in log-log coordinates
42	1—10	44	3,82	0,84	1,66	0,85
45	1—10	50	2,57	0,96	1,17	0,94
47	1—10	51	4,19	0,96	1,39	0,95
48	1—10	100	6,14	0 94	1,42	0,95
49	1—10	91	4,39	0,79	1,38	0,87
50	1—10	100	2,99	0,94	1,42	0 97
51	1—10	100	2,41	0 97	1,34	0,96
60	1—10	39	1,72	0,94	0,72	0,93
64	1—10	44	2,75	0,95	1,45	0,96
71	1—10	187	2,41	0,82	1,11	0,85

MAGNITUDE ESTIMATION

Inspiration

1	1—10	98	3,68	0 96	1,34	0,94
2	1—45	110	38,68	0,92	1,98	0,91
3	1—6	50	3,62	0,92	1,07	0 90
5	1—8	80	5,23	0,85	1,88	0,88
7	1—6	62	4,03	0,87	1,31	0,89
9	1—10	63	5,06	0,85	0,95	0,86
11	1—12	80	4,45	0,94	1 10	0 94
14	1—10	100	5,37	0 93	1 65	0,94
15	1—13	86	5,21	0,92	0,95	0,92
16	1—20	59	9,01	0,91	1,78	0,92

Expiration

17	2—45	104	23,10	0,80	1,87	0,88
18	1—10	99	2,94	0,88	1,21	0,89
19	1—15	99	4,38	0,96	1,06	0,96
20	1—20	100	8,81	0 92	1,45	0,92
21	1—15	92	3,77	0,95	1,00	0 96
22	1—15	101	2,89	0,96	1,00	0 96
23	1—13	97	3,39	0 89	1,08	0,95
65	1—11	57	3,11	0,94	1,53	0,95
170	1—14	100	2,85	0 98	1,13	0,96
172	1—12	100	2,77	0,92	1,25	0,95

JND

Inspiration

subject and experiment code	scale	number of inspirations and expi rations	regression coefficient in linear coordinates	correlation coefficient in linear coordinates	regression coefficient in semilog coordinates	correlation coefficient in semilog coordinates
31	1—15	60	4,45	0,94	6 54	0,96
32	1—16	64	4,07	0,95	4,95	0 91
33	1—21	84	17,59	0,90	11,58	0,91
35	1—15	60	4,29	0,96	6,34	0,90
36	1—21	62	11,26	0,94	10,39	0,90
Expiration						
30	1—21	82	6,11	0 93	6,51	0,91
38	1—17	65	3,23	0 97	5,32	0 91
39	1—15	58	3,78	0,93	6,62	0,87
40	1—16	62	3,75	0,94	6,26	0,87
56	1—21	79	6,94	0 94	10 66	0,90

TABLE 3. Numerical values of individual regression coefficients and Pearson correlation coefficients in EMG registration

CATEGORY PRODUCTION

EMG

subject code	scale	number of inspiration	frequency				number of EMG impulses			
			regression coefficient in linear coordinates	correlation coefficient in linear coordinates	regression coefficient in log-log coordinates	correlation coefficient in log-log coordinates	regression coefficient in linear coordinates	correlation coefficient in linear coordinates	regression coefficient in log log coordinates	correlation coefficient in log log coordinates
1	1—6	30	0,12	0 75	1,40	0,79	0,020	0,82	1,51	0,86
2	1—6	56	0,02	0,74	0,49	0,89	0,008	0,72	0,51	0,87
3	1—6	59	0,02	0,69	0,99	0,77	0,007	0,86	1,02	0,88
4	1—5	25	0 16	0,87	2,15	0,89	0 017	0,89	1 41	0,89
5	1—5	49	0,07	0,89	1,04	0,92	0,013	0,90	0,85	0,92
6	1—6	60	0 01	0,77	0,42	0,91	0,005	0,84	0,38	0,95
7	1—6	30	0,04	0,77	0,51	0,54	0,010	0 78	0,75	0 60
8	1—6	57	0,06	0,94	0,96	0,95	0,009	0,88	0,64	0,95
9	1—6	60	0,05	0 93	0,94	0,93	0,010	0,91	0,81	0,92
10	1—6	56	0,07	0,80	0,48	0,79	0,026	0,74	0,49	0,76
11	1—9	42	0,12	0 74	1,18	0,66	0,022	0,75	0,97	0,65
12	1—5	50	0,07	0,77	1,03	0,79	0,023	0,77	0,57	0,77
13	1—4	40	0,06	0,83	0,83	0,81	0 017	0,68	0,81	0,63
14	1—5	49	0,07	0,84	0,76	0,83	0 019	0,81	0 71	0,80

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 421

FATE OF NORADRENALINE IN THE HUMAN FOETOPLACENTAL UNIT

IN VIVO STUDIES ON PLACENTAL TRANSFER, METABOLISM AND DISTRIBUTION IN FOETAL TISSUES

BY

SEPPO SAARIKOSKI

HELSINKI 1974

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 421

FROM THE DEPARTMENT I OF OBSTETRICS AND GYNAECOLOGY UNIVERSITY OF HELSINKI MEDICAL SCHOOL, HELSINKI, FINLAND

FATE OF NORADRENALINE IN THE HUMAN FOETOPLACENTAL UNIT

IN VIVO STUDIES ON PLACENTAL TRANSFER, METABOLISM AND DISTRIBUTION IN FOETAL TISSUES

BY

SEPPO SAARIKOSKI

HELSINKI 1974

Printed in Finland
Oy Weilin+Göös Ab
Tapiola 1974

CONTENTS

INTRODUCTION 5

REVIEW OF THE LITERATURE 7

- Passage of noradrenaline through the placenta, and its metabolism in the placenta 7
- Noradrenaline in foetal tissues 8
- Metabolism of noradrenaline in the foetus 9
- Uptake of noradrenaline and receptor function in the foetus 11
- Catecholamines and foetal haemodynamics under normal condition and in asphyxia 12
- Noradrenaline during pregnancy 14
- Methods for the determination of radioactive catecholamines and their metabolites 15

PROBLEMS AND PURPOSE OF THE STUDY 18

MATERIAL AND METHODS 19

- Foetuses 19
- *Anaesthesia* 19
- Delivery of the foetus 19
- Administration of radioactive substances 21
- Chemicals 22
- Extraction 23
- Paper chromatography procedure 23
- Determination of total activity noradrenaline and its metabolites 24
- *Determination of conjugated metabolites* 26
- Statistical treatment 27
- Reproducibility and reliability of the method 27
- Comment 29

RESULTS 31

- I Rate of passage of ^{3}H-noradrenaline and its metabolites through the placenta 31
  - Material and methods 31
  - Results 31
  - Comment 33
- II Distribution of ^{3}H-noradrenaline and its metabolites in foetal tissues after i.v. administration of ^{3}H-noradrenaline to the mother 36
  - Material and methods 36
  - Results 36
  - Comment 48

III Metabolism of ^{3}H-noradrenaline in the foetoplacental unit 48
Material and methods 48
Results 49
Comment 56
IV *Distribution and metabolism of ^{3}H-noradrenaline in the human foetus after removal from the uterus* 56
Material and methods 56
Results 57
Comment 61
DISCUSSION 62
Paper chromatography and oxidation method 62
Influence of asphyxia on the results 64
Passage of noradrenaline and its metabolites into the foetus 65
Noradrenaline in the blood during pregnancy as compared with the foetal values 68
CONCLUDING REMARKS 69
SUMMARY 70
ACKNOWLEDGEMENTS 73
REFERENCES 75

ABBREVIATIONS

A	–	Adrenaline
CA	–	Catecholamine
COMT	–	Catechol -O- methyl transferase
CR	–	Crown rump length
DA	–	Dopamine
DHMA	–	3,4-dihydroxymandelic acid
DHPG	–	3,4-dihydroxyphenylglycol
DOPA	–	3,4-dihydroxyphenylalanine
DOPAC	–	3,4-dihydroxyphenylacetic acid
HVA	–	Homovanillic acid
MAO	–	Monoamine oxidase
MHPG	–	3-methoxy-4-hydroxyphenylglycol
MN	–	Metanephrine
MT	–	3-methoxytyramine
NA	–	Noradrenaline
NMN	–	Normetanephrine
VMA	–	Vanillylmandelic acid (3-methoxy-4-hydroxymandelic acid, MOMA)
VA	–	Vanillic acid

INTRODUCTION

The adrenergic nervous system plays a central role in the regulation of the foetal circulation under normal conditions and in foetal adaptation to stress, e.g. to asphyxia, as well as, finally in the adaptation of the newborn to extrauterine life (Dawes 1968, Rudolph and Heymann 1973)

High NA levels in the maternal peripheral blood are considered harmful for the foetus since they may increase uterine contractions and contract the uterine blood vessels, and thus impair the supply of blood to the foetus (Dornhorst and Young 1952, Beard 1962) This phenomenon is believed to be, at least partly responsible for the high perinatal mortality of children of pheochromocytoma patients (Blair 1963 Thiery Derom van Kets, de Schaepdryver Bernard, Bekaert, Hooft, Derom, Rolly and Roels 1967 Batts, Tchilinguirian and Passmore 1974) Similarly those CA s which cross the foetoplacental barrier from the maternal circulation may interfere with the shunts regulating the foetal circulation and could possibly impair the adaptation of the foetus to a crisis situation (Owman, Aronson, Gennser and Sjöberg 1973)

Our knowledge of the functioning of the adrenergic nervous system in the human foetus is based mainly on *in vitro* studies. on determinations of the CA content of foetal tissues, histological and electron microscopic studies and demonstrations of enzyme and receptor activities. The dynamic behaviour of the mediator substance of the adrenergic nervous system, NA, and its metabolites in the maternal placental foetal system *in vivo* is, however poorly understood. Such an understanding is essential if the role of the adrenergic nervous system of the human foetus under normal conditions and in those deviating from normal is to be completely elucidated.

Passage of noradrenaline through the placenta, and its metabolism in the placenta

Placental transfer It has been shown that radioactive NA can traverse the placenta of sheep and guinea-pigs to some extent (Condorelli and Cosmi 1970 Cosmi and Condorelli 1973, Morgan, Sandler and Panigel 1972) but not that of rats or dogs (Mirkin, unpublished data 1971 ref. Mirkin 1975) The passage of NA across the human placenta was thought possible after intravenous administration of CA to mothers, on the basis of indirect indications gained by monitoring foetal heartbeat or foetal glucose and free fatty acid levels (Beard 1962, Zuspan, Whaley Nelson and Ahlquist 1966) but reports contradicting these results have also appeared (Pardi, Tiengo, Uderzo, Salmoiraghi and Candiani 1969)

After injection into mothers, ^{14}C-NA and ^{14}C-A have been shown to pass into unviable foetuses in demonstrable quantities, within 2 to 5 min. The ^{14}C-NA in the umbilical blood of two foetuses in the second trimester was 2 % and 18 % respectively of the maternal plasma concentration (Sandler Ruthven, Contractor Wood, Booth and Pinkerton 1963 Sandler Ruthven and Wood 1964)

Metabolism in the placenta. NA metabolism in the placenta is catalyzed both by the action of MAO and COMT (Pesce and Casmone 1947 Luschinsky and Singher 1948, Thompson and Tickner 1949 Ismalo and Castrén 1967 Klinge 1968) The very high placental MAO activity has been observed to decrease towards the end of the pregnancy with decreasing partial oxygen pressure, and also in toxaemia (Sandler and Coveney 1962 de Maria 1964 Nowack 1966, Sagone and Arrotta 1966) In toxaemia, the MAO activity was not considered lowered unless the placenta was degenerated (Klinge, Penttilä and Tissari 1964 Castrén and Saarikoski 1969)

In vitro in placental homogenates and during placental perfusion, NA is metabolized mainly by the action of MAO (Castrén, Kaartinen and Saarikoski 1968, Morgan et al. 1972 Castrén and Saarikoski 1974) During transplacental passage in guinea-pig and in man, the *in vivo* metabolism also appears to be mediated through both MAO and COMT action (Morgan et al. 1972, Saarikoski 1972)

The NA concentration in the umbilical blood of children of pheochromocytoma patients is only about 10 % of the maternal level. The VMA in the umbilical blood of one foetus was five times higher than in the mother's peripheral blood. This would

also suggest that NA is metabolized during its transplacental passage (Barzel, Bar Ilan, Rumney Lazebnik, Eckerling and de Vries 1964 Bekaert, Barbier van Germeersch and Thiery 1966)

There is no information available on the conjugation of NA and its metabolites in the placenta. Conjugation is unlikely since no p-nitrophenol-sulphatizing or o-aminophenol-conjugating UDPG-hydrogenase or glucuronyl transferase activity is found in the placenta, (Hartiala and Pulkkinen 1955, Dutton 1959 Pulkkinen 1963)

Noradrenaline in foetal tissues

In order to assess the functional status of the adrenergic nervous system in the foetus, it is necessary to estimate the foetus ability to synthetize, store, inactivate and release transmitter substances from the nerve endings.

Peripheral tissues. The CA content of many adrenergically innervated tissues, such as the heart, is low in chicken embryo (Ignarro and Shideman 1968a) and in the foetuses of several mammals (Glowinski, Axelrod, Kopin and Wurtman 1964 Iversen, de Champlain, Glowinski and Axelrod 1967 Friedman, Pool, Jacobowitz, Seagren and Braunwald 1968, Lipp and Rudolph 1972) The CA content of peripheral foetal tissues conforms to the ontogenetic principle that in those foetuses which are well developed at birth there is a higher content than in those which are less well-developed.

In human foetal heart no adrenergic innervation is histochemically demonstrable before the twelfth week of gestation, or in the bowel before the ninth to tenth week (Gennser and Nilsson 1970 Read and Burnstock 1970) As early as the second trimester however the NA content of the heart and many peripheral tissues in the human foetus reaches levels similar to those found in adult tissues of many species (Greenberg and Lind 1961) Using histochemical procedures the human foetal circulatory shunts have been observed to be adrenergically innervated (Boréus, Malmfors, Mc Murphy and Olson 1969, Aronson, Gennser Owman and Sjöberg 1970) The adrenergic innervation of the umbilical cord observable by fluorescence histochemistry extends only about 1 cm outside the body (Boréus *et al.* 1969)

Brain. The NA content in the foetal brain also appears to conform to the ontogenetic principle. In rat brain the NA content three days before birth is only 15 % of the adult level, while in the guinea-pig, which is considerably more mature at birth, cerebral NA concentrations have already attained adult levels at this time (Kärki, Kuntzman and Brodie 1962)

The cerebral NA content is low in human foetuses during the first half of pregnancy (Bertler 1961 Greenberg and Lind 1961 Hyyppä 1972) CA fluorescence was observable in the human foetal hypothalamus from the tenth week of pregnancy and in the median eminence from the 13th week. The DA concentration in the hypothalamus is already clearly higher than the NA concentration during the 11th to 15th weeks of pregnancy (Hyyppä 1972) The topography of the monoaminergic paths in the brain of the human foetus during the second trimester is in its main parts consistent with that observed in the rat 1–2 weeks after birth (Nobin and Björklund 1973)

Chromaffin tissue The chromaffin tissue in the foetus matures at an earlier stage than the adrenergic postganglionic nerves. Primitive sympathetic cells may already be found in human foetuses of 14–16 mm length, and pheochromocytes proper in foetuses 27–46 mm long (Coupland 1952) According to Hervonen (1971) differentiation of primitive sympathetic cells in human foetal aortic paraganglia (Zuckerkandl 1901) and adrenal medulla occurs during the eighth week of gestation.

During the foetal period, the adrenal medulla and sympathetic paraganglia carry the greater part of the total CA content of the foetus and a relatively greater proportion than in adult life (Hökfelt 1951 Shepherd and West 1951 1952 Comline and Silver 1966) The NA concentration in the aortic paraganglia is higher than in the adrenals (Niemineva and Pekkarinen 1952, Coupland 1953)

Towards the end of the intrauterine period the A/NA ratio increases in the adrenal medulla. The adrenal cortical hormones, hydrocortisone in particular are thought to regulate the A synthesis (Roffi 1965 Margolis, Roffi and Jost 1966, Wurtman and Axelrod 1966) The human foetal adrenal medulla is capable of methylating NA to A already during the first half of gestation (Niemineva and Pekkarinen 1952, Greenberg and Lind 1961 v Studnitz 1968)

The sympathetic paraganglia, on the other hand, contain NA, almost exclusively throughout the foetal period (Greenberg and Lind 1961 Brundin 1965, Gennser and v Studnitz 1969b)

Metabolism of noradrenaline in the foetus

The metabolic inactivation of CA is achieved mainly through the action of MAO and COMT and conjugating systems (for ref., see Axelrod 1965 Iversen 1967 Sharman 1973)

MAO In 1928 Hare described an enzyme which oxidized tyramine. It was later found that this enzyme was also responsible for the decomposition of A and NA in rat, guinea-pig and rabbit liver (Blaschko, Richter and Schlossmann 1937a) MAO is present in most tissues but it is particularly abundant in the liver intestine, kidneys and central nervous system (Blaschko, Richter and Schlossmann 1937b) The MAO distribution in the human differs from that in many animals in that human heart displays high MAO activity compared to the lower levels seen in many other species (Langemann 1944 Levine and Sjoerdsma 1962)

Many foetal tissues display low MAO activity In the chicken embryo MAO activity is measurable from the fourth day of gestation with the highest levels being t tained on the 19th day (Ignarro and Shideman 1968b) The MAO activity in rat foetuses during the foetal and early neonatal period is only about 20 % of adult values (Glowinski et al. 1964 Robinson 1967) The MAO activity in rabbit liver as late as two weeks postnatally only amounts to about 10 % of the adult level (Bojanek, Bozkowa and Kurzepa 1966) On the 25th day of pregnancy the MAO activity in guinea-pig kidney liver and intestine is only 10 to 20 % while that in brain is already some 60 % of adult values. The hepatic and intestinal MAO activities subsequently increase rapidly but that of the kidneys increases more slowly At birth, however the MAO activity in all the tissues mentioned is about 60 % of the corresponding adult value (Tiwari 1966)

The human foetal MAO activity has also been considered low compared with that of the adult. The foetal hepatic MAO activity during the first to second trimesters is about 1/4 that of term placenta, which is known to be of the same order as the hepatic MAO activity (Luschinsky and Singher 1948, Davison 1958, Castrén and Saarikoski 1969). In homogenates and mitochondria of the human foetal liver cells MAO activity is constant from the 13th to 28th week of gestation and doubles during weeks 28 to 40 of development (Skorobohata and Drel 1973). In the aortic bodies and in the ductus venosus higher MAO activities than in the liver have been recorded, and in the umbilical blood vessels equal or lower activities (Gennser and v. Studnitz 1969a, 1969b). In human foetal brain MAO activity is demonstrable from the 10th to the 12th week of pregnancy onwards (Himberg and Solatunturi, personal communication 1973).

COMT. COMT is an enzyme responsible for 3-O-methylation of NA and other catechols (Axelrod 1957). It catalyzes the transfer of a methyl group from S-adenosylmethionine to meta-hydroxyl groups in these substances. COMT occurs in a number of tissues, in particular in the liver and kidneys (Axelrod and Tomchick 1958, Axelrod, Albers and Clemente 1959).

The COMT activity of foetal tissues develops in a fashion similar to MAO activity: in the chicken embryo it is observable from the fourth day of incubation. (Ignarro and Shideman 1968b). The COMT activity of the newborn rat is about 20 % of the adult value (Glowinski *et al.* 1964).

In the human foetal heart, adrenals, liver and brain *in vitro* NA metabolism has been found to be mediated mainly by action of COMT. A similar situation also prevails *in vivo* in a number of foetal tissues during the second trimester (Castrén *et al.* 1968, Castrén and Saarikoski 1969, Saarikoski and Castrén 1971). In the foetal umbilical blood vessels or in the aortic bodies no COMT activity is demonstrable at the 12th week of gestation while in the liver the activity is already fairly high (Gennser and v. Studnitz 1969a, 1969b).

On the basis of evidence obtained from post-mortem specimens it is considered that the COMT activity increases with increasing age: in a second trimester foetus the activity is about 13 % of that in the neonate and about 1.3 % of the activity in a 50-years-old person (Agathopoulos, Nicolopoulos, Matsaniotis and Papadatos 1971). Newborn excrete mainly O-methylated catecholamines and VMA in their urine in amounts which increase during the first days after birth, but more distinctly in premature than in full-term infants. This situation was considered to indicate a deficiency in oxidative deamination activity at birth and, on the other hand, increasing COMT activity during the immediate neonatal period (Brunjes, Castner and Hodgman 1964, Nicolopoulos, Agathopoulos, Danelathou-Athanasiadou and Bafataki 1968).

Conjugation with glucuronic or sulphuric acid. Some A, NA, and CA metabolites are excreted in urine conjugated with glucuronic or sulphuric acid (Richter 1940, v. Euler, Hellner-Björkman and Orwén 1955, Elmadjian, Lamson and Neri 1956, La Brosse, Axelrod, Kopin and Kety 1961, Haggendal 1963b, Goodall, Alton and Rosen 1964). The liver and intestine are thought to be the most important sites of conjugation, but conjugation also takes place in other tissues. The principal metabolite found in rat brain after intracisternal administration of ^{3}H-labelled NA and NMN

was MHPG-sulphate (Schanberg, Schildkraut, Breese and Kopin 1968) In human spinal fluid MPHG was also present as its sulphate conjugate (Schanberg, Breese, Schildkraut, Gordon and Kopin 1968)

There is no positive indication of CA conjugation in the human foetus. Conjugation appears possible however since after H NA administration to the mother nearly 1/3 of the metabolites in the foetal liver were conjugates, conjugated NA, NMN and MHPG-VMA were detected (Saarikoski 1972) In the liver and other tissues of rabbit and human foetuses the activities of the glucuronidating enzyme system is low towards both p-aminophenol and steroids (Hartiala and Pulkkinen 1955 Dutton 1959) In contrast p-nitrophenol, phenol and steroid sulphate ester formation has been demonstrated in human foetal liver lungs, adrenals, kidneys and intestine (Pulkkinen 1963 1966, Wengle 1964)

Uptake of noradrenaline and receptor function in the foetus

Uptake The machinery required for the storage and binding of endogenously synthesized and exogenously administered CA appears to be poorly developed during the foetal period. Chicken embryotic heart can bind H NA from the fifth day that is from the time adrenergic innervation is histochemically detectable (Ignarro and Shideman 1968c) H NA binding in the rat heart and spleen increases significantly only 2 to 3 weeks after birth, whereas intestinal and salivary gland NA binding capacity is the same at birth as in the adult (Glowinski *et al.* 1964 I eisen *et al.* 1967)

Human foetal intestine can bind NA *in vitro* before innervation can be visualized by fluorescence histochemistry (Read and Burnstock 1970) In the intact human foetoplacental unit *in vivo* H NA uptake by the foetal tissues appears comparatively poor (Saarikoski and Castrén 1971)

Receptor function. According to classical receptor theory two kinds of adrenergic receptors exist: adrenergic alpha receptors, stimulation of which usually produces an excitatory response in the cell, while stimulation of beta receptors most often results in an inhibitory response (Ahlquist 1948)

Frequent attempts have been made to study the function of the foetal adrenergic nervous system by determining the effect of adrenergic alpha and beta agonists and antagonists in various foetal tissues. The adrenergic receptors are believed to function at an early stage of foetal development, in animal as well as human foetuses. NA is thought to increase the cardiac frequency of the rat foetus *in vitro* prior to the 16th postcoital day but not after that, while A has a similar effect after but not before the 16th day (Bernard and Gargouil 1967)

In the human foetal heart, *in vitro*, A causes a chronotropic and ionotropic response already in the 9th or 10th week of pregnancy that is before the adrenergic innervation of the heart is histochemically demonstrable (Genmser and Nilsson 1970) In addition, from the 12th week the foetal cardiac response to A is comparable to that of the adult (Baker 1953) In the human foetal small intestine beta receptor function is clearly demonstrable *in vitro* from the 11th to the 23rd week of pregnancy (McMurphy and Borëus 1968) According to other authors, t this time alpha receptor function is also observable in human foetal small intestine (Hart and Mir 1971a) as well as

in the colon, where stimulation of alpha-adrenergic receptors causes contraction and that of beta-adrenergic receptors relaxation (Hart and Mir 1971b)

In the human foetus adrenergic receptor function is also demonstrable in the ductus arteriosus during *in vitro* perfusion and using the spiral strip experimental arrangement (Boréus *et al.* 1969) The results suggest that adrenergic mechanisms participate in the closure of the ductus arteriosus. NA, acetylcholine and serotonin also participate in the contraction of the intra-abdominal part of the umbilical vein and the initial part of the ductus venosus in the human foetuses of 20th to 24th weeks. A NA-like response can also be elicited with tyramine and can be inhibited with phenoxybenzamine, which blocks the alpha-adrenergic receptors. These results support the theory which proposes that a functional adrenergic sphincter mechanism exists at these points (Ehinger Gennser Owman, Persson and Sjöberg 1968 Owman *et al.* 1973)

Catecholamines and foetal haemodynamics under normal conditions and in asphyxia

Normal situation. The greater part of our knowledge of foetal haemodynamics is based on animal experiments either with exteriorized foetuses or with so-called chronic preparations. Although activity of the adrenergic receptors of the end organs is demonstrable at an early stage of foetal development, rather little investigation of the contribution of the adrenergic nervous system to the regulation of the vasomotor tonus of the blood vessels, the cardiac frequency and the contraction force of the heart in the foetus has been made.

A injected into the rat foetus causes an increase in blood pressure and pulse pressure and bradycardia, injection into the mother results in lowered foetal blood pressure and pulse pressure and bradycardia (Girard 1965) NA and A injected directly into the foetal circulation of the sheep towards the end of pregnancy increase the contraction force of the heart, the carotid circulation and the blood pressure, from which ensues reflectory bradycardia (Pardi *et al.* 1969) According to the same authors the sensitivity of the foetus to these CA s is less than that of the adult, and it is lower at the beginning than at the end of the foetal period.

In sheep foetuses prepared for chronic experiments at a gestational age of 85 to 150 days, atropine and the alpha receptor-blocking agents phenoxybenzamine and phentolamine raise the heart frequency of the foetus even in the case of younger foetuses, which shows that both cholinergic and adrenergic regulation are active in the foetus (Vapaavuori Shinebourne Williams, Heymann and Rudolph 1973)

Using an *in utero* preparation and the microsphere and antipyrine method for studying the circulation and its distribution (Rudolph and Heymann 1967a, 1967b) beta adrenergic receptor stimulation with isoprenaline was found to increase the heart frequency cardiac output, umbilical circulation and coronary circulation in the sheep foetus. On the other hand, there was no change in the proportion of the cardiac output received by other organs, nor did the blood pressure increase. In contrast, alpha-adrenergic receptor stimulation with methoxamine increased the blood pressure of the foetus and slightly reduced the cardiac output, the umbilical circulation, myocardial circulation and renal circulation (Rudolph 1969 Barrett, Heymann and Rudolph 1972, Rudolph and Heymann 1973)

The cortical activity of the brain also appears to influence the circulation of the sheep foetus *in utero*. The change in the EEG (brain curve) from the slow high volt age curve to the fast low voltage curve, which occurs during REM (rapid eye movement) sleep, is associated with a simultaneous irregular respiratory activity and an increase in the heart frequency and blood pressure (Dawes, Fox, Leduc, Liggins and Richards 1972)

It is not possible to perform similar chronic experiments with human foetuses: as a rule, all experiments that have been carried out were acute and asphyxia was a remarkable factor in them. NA causes an elevation of the blood pressure and increased heart frequency in the exteriorized second trimester human foetus (Enhörning and Westin 1954) Close to the end of pregnancy the human foetal heart appears to function with the same autonomous regulatory mechanisms as that of the adult (Renou, Newman and Wood 1969)

Asphyxic situation. The cardiovascular response to hypoxia appears to be associated with the functioning of the autonomous nervous system. In the sheep foetus an increase in systemic blood pressure, vasoconstriction and an increase in heart frequency in response to hypoxia are seen in 70 to 80 day of foetuses, that is at the time when the first response is elicited by stimulating the vagus nerve or the adrenergic nerves (Born, Dawes and Mott 1956, Dawes 1968)

In the newborn rabbit severe asphyxia results in a considerable depletion of the CA content of the extra-adrenal chromaffin tissue (Brundin 1966) In the human foetus, asphyxia of 10 to 15 min duration causes a significant decrease in CA fluorescence in the aortic bodies, but only a slight change in the CA fluorescence of the adrenal medulla (Hervonen and Korkala 1972) It has been suggested that during the foetal period functioning secretory innervation is probably lacking in the extra-adrenal chromaffin tissue. Thus NA would be liberated from this tissue primarily under the influence of chemical and humoral stimulation in response to asphyxia, wherein this tissue is thought to be of considerable significance (Lempinen 1964 Brundin 1966, Hervonen 1971 Hervonen and Korkala 1972)

Both sheep and bovine foetal adrenal medulla also contains cells which respond directly to asphyxic situations by releasing NA. These cells are sensitive to decreases in pO_2, but not to increased pCO_2: lowering of the pO to 4—5 mmHg causes NA release. Bovine foetal adrenal gland ppears to contain only cells of this type. The innervated part of the sheep foetal adrenal medulla reacts in a similar manner to decreases in pO_2. NA secretion increases when the pO goes down to 12—16 mmHg and that of A when the pO decreases to 8—12 mmHg (Comline and Silver 1961 1966, Comline, Silver and Silver 1965) Closure of the umbilical cord causes increased foetal blood pressure; the beta-adrenergic receptor blockers react to prevent this increase, and thus results in circulatory collapse (Joelsson and Barton 1969)

If the CA stores in a guinea-pig mother and foetus are completely emptied by administering reserpine, the incidence of perinatal mortality increases. Under such conditions the newborn cannot endure the postnatal asphyxia (Towell, Hyman James, Steinsland, Gerst and Adamsons 1965) Brief exposure to reserpine does not impair the response of newborn guinea pigs to asphyxia (Towell 1971) An intact functioning autonomous nervous system appears to be indispensable for the foetus successful adaptation to and management of asphyxia.

The foetal heart tolerates anoxia considerably better than that of the adult, and under *in vitro* conditions the human foetal heart is superior to that of many other species in this respect (Penn 1970) Adrenaline impairs the recovery of the foetal atrium from anoxia. The beta-adrenergic receptor blocking agent propranolol inhibits the effect of adrenaline (Penn 1970)

Effect of exteriorization. Some 40 to 50 % of the sheep foetal cardiac output goes to the placental circulation. The proportion received by the placenta from the human foetus is thought to be of the same order When the foetus is removed from the womb the placental circulation rapidly abates. Within 30 min the umbilical circulation of the exteriorized human foetus reaches nearly unmeasurable levels (Heymann and Rudolph 1967 Rudolph, Heymann, Teramo, Barrett and Räihä 1971) The blood gas values: the pH, pCO and pO in the umbilical blood vessels remain normal, but the peripheral blood gas values in the human foetus rapidly change towards acidosis: the pO decreases, the pH decreases and the pCO_2 increases. Since the umbilical circulation is reduced, the distribution of the circulation in the foetus also undergoes a change: the brain the coronary circulation nd the adrenals begin to receive more blood (Rudolph *et al* 1971) The results of earlier studies on experimental animals were for the most part similar (Barker 1966, Campbell, Dawes, Fishman and Hyman 1967 Purves and James 1969)

Noradrenaline during pregnancy

NA and A disappear rapidly from the circulation as a result of tissue uptake (Pekkarinen 1948, Lund 1951 Axelrod Weil-Malherbe and Tomchick 1959 Hertting, Axelrod and Whitby 1961 Iversen 1967) The half life of NA and A in human blood was determined to be 0.5 to 2.3 min (Cohen, Holland, Sha and Goldenberg 1959 Vendsalu 1960)

During pregnancy the disappearance of ^{14}C-labelled dl-NA and dl-A does not differ from that in the non-pregnant subject. This is also true for H-labelled l NA (Sandler *et al* 1964 Saarikoski, unpublished data 1972) In addition, the urinary excretion of NA, A and VMA does not change during pregnancy (Castrén 1963 Zuspan, Nelson, Ahlquist and Williams 1967 Pekkarinen and Castrén 1968) Only on the day following parturition is the excretion of NA seen to be significantly elevated (Zuspan 1970 Goodall and Diddle 1971)

The female reproductive organs are adrenergically innervated. The NA concentration is highest in the ovaries and lowest in the fundal part of the uterus, where it is only about 1/4 of that in the cervix (Anton and Sayre 1962 Gaffney Burket and Woronkow 1965, Owman, Rosengren and Sjoberg 1967) Uterine NA concentrations increase in a number of species at the beginning of pregnancy but decrease strongly towards the end of the pregnancy (Laes, Pekkarinen, Saarikoski and Suramo 1967 Sjöberg 1967) At present the function of the adrenergic nervous system in the puerperal organs is incompletely understood.

Methods for the determination of radioactive catecholamines and their metabolites

A number of methods have been described for the separation and physicochemical determination of CA's and their metabolites which have also been applied to the determination of C- and ^{3}H labelled A, NA and their metabolites.

Extraction from tissues

Various agents have been used to extract CA's and their metabolites, for example, trichloroacetic acid, ethanol, hydrochloric acid, formic acid perchlorid acid. Of these, 0.4 mol/l perchloric acid is the most widely used and it is considered best by many workers (Bertler Carlsson and Rosengren 1958, Anton and Sayre 1962, Rentzhog 1972)

Isolation and purification

Column chromatography

Aluminium oxide column A widely used technique for CA isolation involves adsorption to aluminium oxide. Aluminium oxide binds CA's well at alkaline pH's (pH 8—8.5) but not at acidic pH's (Shaw 1938) The adsorption is specific for catecholamines and their catechol metabolites: DOPA, DA, A, NA, DHMA, DHPG DOPAC; O-methylated metabolites: MN NMN MHPG and VMA remain in the effluent. Subsequent elution is usually performed with acetic acid, formic acid or hydrochloric acid. Recovery varies from 60 to 80 %

This procedure has also been employed for the isolation of radioactive CA's (*e.g.* Axelrod, Weil-Malherbe and Tomchick 1959 Sandler *et al.* 1963, 1964) The method however only provides block separation of catechol and O-methylated metabolites. If it is desired to determine NA and its principal metabolites individually additional separation techniques must be employed.

Ion exchange columns. Bergström and Hansson (1951) used an Amberlite IRC-50 column to separate NA and A. This resin has also been used in the determination of urinary catecholamines and their alkaline metabolites (Kirschner Goodall and Rosen 1959) The strongly acid resin, Dowex 50 is widely used for extracting CA's from tissue extracts (Bertler *et al.* 1958 Häggendahl 1962 1963a, Iversen 1963) This resin will adsorb CA's and their O-methylated metabolites at neutral pH. Under these conditions the catechol metabolites go into the effluent. If a long Dowex column is used DA, NA, A, NMN and MN can be eluted separately (Häggendahl 1962, Iversen 1963) The recovery with this method, with physicochemical determination, is as high as 85 to 95 % Dowex-50 is also widely used in the determination of radioactive NA and A and of their metabolites. However it is laborious and slow In addition, the separation of NA and all its metabolites cannot be achieved with this method alone.

Aluminium oxide columns and ion exchange resins used in succession. In 1961 Kopin, Axelrod and Gordon described a method which is now widely used for separating and determining radioactively labelled catecholamines and their individual metabolites. In this procedure, both aluminium oxide columns and Dowex 50 or Amberlite CG-50 columns are used. The free catechols are adsorbed onto aluminium oxide (Weil-Malherbe and Bone 1952) and eluted with 0.2 mol/l HCl. The conjugated catechol derivatives and O-methylated metabolites pass through with the effluent and washings. The deaminated catechols DHMA and DHPG are extracted into ethyl acetate from an aliquot of the eluent acidified with 6 mol/l HCl and saturated with NaCl. The conjugated catechols are extracted from an aliquot of the aluminium oxide effluent after acid hydrolysis.

The pH of the remainder of the aluminium oxide effluent is adjusted to 6.5 with 1 mol/l HCl and passed through a Dowex 50 ion exchange column. NMN and the other methoxyamines are eluted from the column with 3 mol/l NH OH. VMA and MHPG pass in the effluent. VMA is extracted from the effluent with ethyl acetate, after adjusting the pH to 1 with 6 mol/l HCl. MPHG is then extracted from the Dowex effluent, after saturation with NaCl, with ethyl acetate.

The MN NMN and MHPG conjugates are enzymatically cleaved and determined. A similar method has also been published by Masuoka, Drell, Schott, Alcaras and James (1963) These methods have the drawback of polytomy and slow and laborious execution. Thus their application is restricted if the number of samples is large. Furthermore, the recoveries of different metabolites may vary considerably which impedes the interpretation of results.

Thin layer chromatography

Thin layer chromatography has not been used to the same extent as column chromatography in the determination of radioactive CA s. Chromatography on cellulose layers with n-butanol.3 mol/l HCl as solvent separates several CA s and CA metabolites (de Potter Vochten and de Schaepdryver 1965) as does chromatography on polyamide plates with isobutanol:acetic acid cyclohexane (80·70·10) as solvent (Segura-Cardona and Soehring 1964) To the author's knowledge these methods have not been used for radioactive CA determination. Rentzhog (1972) has described several solvent systems for use with silica gel layers for determining acetylated CA s. Glese Rüther and Matussek (1967) used cellulose plates with a modification of the Partridge solvent system for separating and quantitatively determining radioactive NA and its metabolites. Extraction was performed with 0 4 mol/l perchloric acid. After centrifugation, the supernatant was neutralized and applied in drops as a band across the plate. After development, the activity in the areas consistent with each metabolite was determined by scraping them into scintillation flasks. The recovery was about 96 for all metabolites. Thin layer chromatographic procedures are fast and comparatively simple However the limited quantity of the aliquot that can be applied to each plate must be considered a drawback, as is the comparative unsuitability of the thin layer for combustion.

Paper chromatography

Paper chromatography has not been widely used in the quantitative separation of radioactively labelled CA s, although such procedures involving the use of various solvent systems have been employed for both qualitative and quantitative CA sepa ration. The phenol. water system has been used to separat both acetylated and unacetylated CA metabolites and amines (James 1948, Crawford 1951 Goldstein, Friedhoff and Simmons 1959) The most widely used system for separating catecholamines and their metabolites (James and Kilbey 1950) is Partridge s solvent (butanol.acetic acid:water 4 1.5 or 4 1:1 or 4 1 2) (Partridge 1948) Schanberg *et al.* (1968b) used two different solvent systems butanol ethanol water and Partridge s sol ent, in their studies of the metabolism of H-NMN n rat brain. After p-nitroaniline staining, strips 1 cm in width were placed in a phosphor:ethanol (9 1) solution and the tritium was determined by liquid scintillation counting, thus providing a quantitative paper chromatographic method. Many workers use paper chromatography qualitatively in association with quantitati e CA analysis based on column chromatography etc. as an additional parameter for identifying the radioactive peak with known metabolites (*e g* Kopin *et al.* 1961 LaBrosse *et al.* 1961) Paper chromatography has also been used in the examination of the purity of H-labelled catecholamines.

Rutschmann, Pacha, Kalberer and Schreier (1965) ha e described a method based on two-dimensional paper chromatography for studying catecholamine metabolism. They used Whatman no. 1 paper and n-butanol saturated with 1 mol/l HCl (v Euler and Hamberg 1949) and nitroethane:70 % acetic acid (9·4) as solvents. W th this method they were able to determine ten different catecholamines and catecholamine metabolites: DOPA, DA, NA, A, MT NMN MN DHMA, VMA and HVA.

CA s and thei metabolites can be efficiently separated using paper chromatography but elution of these substances from the paper for further physicochemical or radiochemical analysis has many attendant difficulties. However when paper chromatography is coupled with the incineration method the problems of elution are over come, and a good counting efficiency is obtained.

PROBLEMS AND PURPOSE OF THE STUDY

The adrenergic nervous system plays a central role in the regulation of the foetal circulation under normal conditions and in crisis situations, e g in asphyxia. The information available on NA biochemistry in relation to the foetoplacental unit is sparse and that concerning the dynamic aspects of NA metabolism is totally lacking It has not either been clarified with any certainty whether the NA in the mother's blood affects the foetal circulation or whether the effects induced in the foetus by catecholamine administration to the mother in fairly high doses are merely reflections of actions on the uterine blood vessels and the uterine muscles. In the assessment of the functional status of the adrenergic nervous system in the human foetus in various physiological and pathological situations the main difficulty arises from the scarcity of fundamental data. Additional studies would thus seem essential

The object of the present study was.

1 To develop a simple reliable and fast method for the analysis of radioactively labelled NA and its metabolites under conditions where it is desirable to use small amounts of radioactivity for instance in human experimentation

2 To study the rate of passage of NA and its metabolites through the placenta.

3 *To study the ability of foetal tissues to take up store and metabolize* NA and its metabolites.

4 *To study the ability of the foetus to conjugate* NA and its metabolites.

MATERIAL AND METHODS

Foetuses

The foetuses used in this study were obtained during the legal interruption of pregnancy. None of the patients suffered from any acute physical disease. The majority of the pregnancy interruptions were made for social, and partly with psychiatric reasons. Foetal age was determined from the mother's last menstruation, the size of the uterus, the foetal weight and the CR length (see Hervonen 1971). The material is described in Tables 1 and 2. The duration of pregnancy in the series varied from 11 to 24 weeks and the weights of the foetuses, from 15 to 420 g.

The purpose of the study and the procedures involved were explained to every woman prior to surgery and each subject consented to participate. The proced res associated with the study did not prolong the hospitalisation period and did not impede recovery.

The study comprises four parts, identified in the results section with the Roman numerals I to IV.

Anaesthesia

As premedication, prior to surgery the women were given atropine 0.1 mg per 10 kg i.m. and diazepam 10–15 mg i.m. (in partial studies I and II) or instead of the latter 25 mg prometazine chloride and 50 mg pethidine i.m. (in parts III and IV). Anaesthesia was induced with thiomebumal, which was administered intravenously. The anaesthesia was continued with halothane at concentration of 0.5 % and 2:1 mixture of nitrous oxid and oxygen i order to avoid contraction of the uterus prior to sampling. For intubation, the patients were given succinylcholine chloride as a muscular relaxant, followed by d-tubocurarine or alkuron chloride.

Delivery of the foetus

All discontinuations of pregnancy were performed by laparotomy. The bdominal covers were entered through a Pfannenstiel or low transverse, incision. The muscular layer and peritoneum were opened longitudinally. The terus was opened by trans-

TABLE 1 *Age and weight of foetuses.*

No.	Weight, g	CR cm	Week of gestation	Partial Study
1	15		11	III
2	16	5.9	12	II
3	10	7.2	13	II
4	20		12	III
5	22	6.6	12	II
6	33		13	III
7	35	8.5	14	IV
8	42	8.7	14	II
9	42	9.4	14	II
10	47	9.5	14	II
11	48	9.2	14	II
12	58	10.4	15	II
13	60	10.4	15	II
14	60	10.8	15	II
15	60		15	III
16	62		15	III
17	62	9.5	15	II
18	63	9.8	15	II
19	66	10.2	15	IV
20	70	11.4	16	II
21	70		16	III
22	73		16	III
23	74	10.8	16	IV
24	75		16	III
25	76	11.6	16	I
26	85	11.4	16	I
27	86	11.0	16	II
28	87		16	III
29	88	11.6	16	I
30	94	11.7	16	III
31	110	12.5	17	II
32	115	11.9	17	II
33	140	14.1	18	I
34	147	14.4	18	II
35	157	14.6	18	IV
36	160	15.1	19	I
37	206	16.9	20	II
38	221		20	III
39	253	16.5	20	III
40	255	16.0	20	IV
41	260	16.2	20	IV
42	274		20	III
43	350	18.6	22	I
44	470	22.0	24	I

TABLE 2. *Age and weight of mothers.* Mothers given ³H-NA into their antecubital vein included.

Age, years	n	Weight, kg	n
<20	5	<50	2
20–30	9	50–60	13
30–40	8	60–70	8
>40	3	>70	2

verse incision in the isthmic part. A sample of the amniotic fluid was taken prior to entering the uterus, and a specimen of uterine muscle from the margin of the uterine incision.

The foetus was always removed from the uterus. Attempts were made to keep the umbilical cord free, and its manipulation was confined to a minimum. When working on the foetoplacental unit the foetus was covered with a cloth saturated with saline for the duration of the experiment.

Administration of radioactive substances

l-Noradrenaline-7 ³H of high specific activity was used in the study except in part III where also dl-noradrenaline 7-³H was employed. The material was in aqueous solution as the acetate and had a specific activity between 5.8 and 8.7 Ci/mmol (obtained from the Amersham Radiochemical Centre, England). The ³H labelled NA diluted with physiological saline was injected either into the mother's antecubital vein (parts I and II), into the umbilical vein of the foetus (parts III and IV) or into the jugular vein of the foetus (part III). The administration of the radioactive substrate and the sampling procedure are described in each part of the study.

In this study the plasma NA concentration at the time of ³H NA injection was not determined, nor was the endogenous NA concentration in the maternal or foetal blood or in the foetal tissues determined in connection with the individual samples. The peripheral NA concentration is believed to reflect the NA overflow from the adrenergically innervated tissues. Changes in plasma NA concentrations are thought to reflect changes in the activity of the adrenergic nervous system in the tissues (see Axelrod 1965, Iversen 1967).

It is known from previous studies that under standardized at rest conditions the changes in plasma NA concentration are comparatively minimal, and fluothane or thiomebumal/nitrous oxide/oxygen anaesthesia does not significantly change the plasma NA concentration even during the operation (Hamelberg, Sprouse, Mahaffey and Richardson 1960, Nikki, Takki, Tammisto and Jaattela 1972). In the last of the studies cited, the mean plasma NA concentration of 11 abdominal surgical patients was 0.66 μg/l and the coefficient of variation was 30.0 %. The variation in the values obtained for replicate samples with the method used was ≤ 10 % (Tammisto, Jaattela, Nikki and Takki 1971). The samples taken from five patients under at rest con-

ditions during pregnancy showed a mean plasma NA concentration of 0.65 µg/l with a coefficient of variation of 33.3 % (Saarikoski, Jäättelä and Ikonen unpublished data 1972)

In the present study the H NA and metabolite concentrations in the foetus and its mother are compared. This reduces the influence of dosage variation on the results. The quantities of blood obtained from the foetuses were so small, from 0.5 to 1.5 ml, that they would not suffice for the determination of the endogenous content of NA and its metabolites by conventional spectrofluorometric methods.

The available counting efficiency for H activity is very low According to the International Commission on Radiological Protection the maximum safe daily dose of H is 1000 µCi. The dosage used here was 50 or 100 µCi, between 1/10 and 1/20 of the latter

The radiation effect produced by the administered radioactivity also depends on the biological half-life of the substance in the organism. The radiation dose can be determined from the formula $D_\beta(\infty) = 73.8 \; C \; \bar{E}_\beta \; T_{ff}$ rad (Johns 1971) where C stands for the activity per unit mass, $\bar{E}_\beta$ for the mean energy of the beta particles, and T_{ff} for the biological half-life of the substance. It was found in the present study that of the H activity administered 43 1 ± 4 1 % was excreted in the urine during the first 24 hours. If the biological half life is taken to be 27 hrs (see Iversen 1967) the patient's weight 60 kg and the dose 100 µCi, the radiation dose with reference to the whole body is D (∞) = 73.8 (100/60,000) 0 006 (27/24) = 0.83 mrad. Such a dose is very small and does not endanger the patient in any way

Chemicals

For treatment of tissues. Perchloric acid (E. Merck AG) l-ascorbic acid (E. Merck AG) ethyl alcohol A7 (Oy Alko Ab) Glusulase® (Endo Laboratories) Ketodase® (Warner Lambert Pharmaceutical Company)

For paper chromatography n-butanol (E. Merck AG) formic acid (E. Merck AG) butyl formiate (Fluka AG) glass distilled water p-nitroaniline (E. Merck AG) K_2CO (E. Merck AG) NaNO (E. Merck AG)

C1 and metabolites l-adrenaline hydrochloride (Fluka AG) l-noradrenaline-d-hydrogen tartrate (Fluka AG) dl-metanephrine hydrochloride (Sigma Chemical Company) 3 4-dihydroxyphenylglycol (Sigma Chemical Company) dl-3 4-dihydroxymandelic acid (Sigma Chemical Company) 3-methoxy-4-hydroxyphenylglycol, piperazine salt (Sigma Chemical Company) dl-3-methoxy-4-hydroxymandelic acid or vanillylmandelic acid (Sigma Chemical Company) 3-methoxytyramine hydrochloride (Sigma Chemical Company) 3 4-dihydroxyphenylalanine (Fluka AG) dopamine (3-hydroxytyramine hydrochloride Fluka AG) vanillic acid (3-methoxy-4-hydroxybenzoic acid Sigma Chemical Company) homovanillic acid (Calbiochem) 3 4-dihydroxyphenylacetic acid dicyclohexylamine salt (Calbiochem)

For liquid scintillation counting dioxane (E. Merck AG) toluene (E. Merck AG) napthalene (E. Merck AG) 2,5-diphenyloxazol (PPO New England Nuclear Chemicals GmbH) P bis[2 (4-methyl-5-phenyloxazolyl)]-benzene (dimethyl-POPOP New England Nuclear Chemicals GmbH) dl-normetanephrine-7 H (in 0.1 mol/l acetic acid, specific activity 3 2 Ci mmol, New England Nuclear Chemicals GmbH)

Extraction

Blood and tissue NA and NA metabolites were extracted with 0 4 mol/l perchloric acid To facilitate staining 1 mg NA 0 5 mg NMN 1 0 mg DHMA and DHPG and 0.5 mg MHPG and VMA were added to the perchloric acid in an ice bath. To the samples from the adrenals and aortic bodies 1 0 mg A and 0.5 mg MN also were added. The perchloric acid contained ascorbic acid (1 mg/10 ml) as an antioxydant.

After placing the tissues and blood in pre weighed tubes containing perchloric acid, they were rapidly cooled in an ice bath by adding ethanol to the crushed ice. This lowers the temperature to between −15 and −20 C. After weighing the tissues were homogenized in an Ultra Turrax (Tp 18/2 Janke & Kunkel KG) homogenizer

Not sooner than 30 min after homogenization the samples were centrifuged for 20 min at 10 000 × g at +4 C and the supernatant collected for the determination of NA metabolites and total activity

The samples were neutralized with 2 mol/l K_2CO_3 (to pH 5 4—5 8) Usually about 70 μl of K_2CO_3 per ml of supernatant was required.

Paper chromatography procedure

The paper chromatographic method employed was developed by the author and has not been published in detail, although it was used in some earlier studies (Castrén *et al* 1968 Castrén and Saarikoski 1969 1974)

Whatman no 1 paper was used The solvent system used was n butanol formic acid butyl formate:water (7 1 1.2 v/v) freshly prepared, or a two-day-old mixture n butanol formic acid water 70·12.15 v/v

An aliquot of the supernatant was spotted dropwise across the lower margin of a paper strip 9—10 × 30 cm and raised as a thin band to a height of about 4 cm from the lower margin with distilled water using the so-called running up technique. The volume of the aliquot spotted was between 0 5 and 2.5 ml, most often 1.5 ml. The paper strips were then suspended in the chromatography tank over the running solvent to equilibrate for 18 to 36 hrs, usually 24 hrs The chromatograms were developed for 12 hrs in an ascending fashion at room temperature. If it was desired to determine NA/A and NMN/MN separately descending chromatography for 18 to 22 hrs was employed

TABLE 3. *Rf values of NA and major metabolites.* Paper: Whatman no. 1, solvent: n-butanol: formic acid: water 0:12:15 v/v, 2 days old, temperature 22° C, equilibration time: 24 hrs, ascending time: 12 hrs.

Compound	Rf value
NA	0.18
NMN	0.34
DHPG	0.49
DHMA	0.56
MHPG	0.68
VMA	0.75

TABLE 4. *Rf values of catecholamines and their metabolites.* See legend to Table 3.

Compound	Rf value
NA	0.18
DOPA	0.19
A	0.23
NMN	0.34
MN	0.38
MT	0.46
DHPG	0.49
DHMA	0.56
MHPG	0.68
VMA	0.75
DOPAC	0.80
HVA	0.90
VA	0.93

The chromatographic strips were dried after the run. They were then stained by spraying with a p-nitroaniline reagent. 1 part 0.1 % p-nitroaniline (100 mg p-nitroaniline solved in 2 ml of conc. hydrochlorid acid and diluted with 98 ml of distilled water) and 1 part 0.2 % $NaNO_2$ were mixed and kept at +4 °C for 5 min and finally 2.5 parts 10 % K_2CO_3 was added immediately before use. The Rf values of the CA and CA metabolites in this system are given in Tables 3 and 4 and the chromatograms are shown in Figs 1 and 2.

NA and the catechol metabolites DHMA and DHPG stain light blue and the methoxy derivatives NMN, MHPG and VMA violet. The metabolites are easily differentiable.

Determination of total activity, noradrenaline and its metabolites

The total ^3H content in the tissues was determined by applying part of the neutralized extract in drops to the lower margin of a Whatman no. 1 paper. After drying, the paper strip was combusted using the sample oxidizer of Kaartinen (1969), and the quantity of ^3H_2O produced was determined by liquid scintillation counting. The scintillation

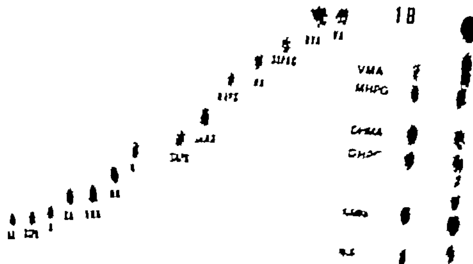


Fig. 1. *Chromatogram [illegible] catecholamines and* [illegible] shows the behaviour of 14 compounds on [illegible] run from separate start point. — Fig. 1B, [illegible] metabolites all applied at the same point. Fig. 1[illegible] applied at the same starting point. Paper [illegible] n-butanol:formic acid: water 0:12.15 [illegible] 2 [illegible] 22 C, equilibration time: 12 hrs. Stained with [illegible]

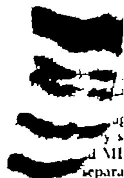


Fig. 2. *Chromatogram of noradrenaline and major metabolites* [illegible] from neutralized placental homogenate was applied [illegible] raised with water to height of about 4 cm. [illegible] solvent: n-butanol: formic acid: butyl formate [illegible] equilibration time: 24 hrs, ascending time: 12 hrs, nitroaniline reagent, NA, DHPG and DHMA are [illegible] and NMN. MHPG and VMA are stained [illegible]

[illegible] d MI [illegible] separa[illegible] method [illegible] ple In a

samples free of activity in alternation. The average retention effect from nine samples and the corresponding blanks was 0 18 ± 0 08 % (mean ± S D) The results are consistent with those reported by Kaartinen (1969)

Chromatography

Recovery of total activity The total activity recovery was determined by adding a known quantity of ³H NA or ³H NMN to a tissue homogenate. After centrifugation, the supernatant was neutralized and applied to the lower margin of the paper and dried The sample was then burned. The activity recovered was compared with that obtained by adding an equivalent amount of radioactivity directly to the scintilla tion liquid The mean of 14 determinations was 100 7 ± 2 1 % (mean ± S D) *It can thus be seen that no activity is lost in the centrifuging* and neutralizing operations.

Recovery of metabolites The *recovery of ³H NA and its metabolites* from the paper chromatography procedure without hydrolysis and after hydrolysis was determined by adding known quantities of ³H NA, ³H *NMN* ³H *DHPG DHMA or* ³H *MHPG-VMA to foetal tissue homo*genates of heart liver placenta, lung brain blood or kidney In some of the tests a mixture of several kinds of tissue was used Since no ³H *labelled DHPG DHMA MHPG* and *VMA were available these were* prepared by incubating l ³H NA or dl ³H NMN with placental homogenates for 15 min as previously described (Castrén and Saarikoski *1969 1974*) The metabolites were isolated and purified using the paper chromatographic process employed in this study and were eluted with 0 1 mol/l acetic acid. The eluates of interest contained ³H DHPG and ³H DHMA in proportions 3 1 and ³H MHPG and ³H VMA in proportions 2 1 respectively For chromatography without hydrolysis the recovery values for NA and its metabolites (94 1—97 4 Table 5) did not differ at any statistically significant level. This was also the case for the recovery values obtained when the same hydrolytic proce dure was included The percentage of the activity spreading out on the paper *ad fundum* is rather equally distributed between the different metabolites No corrections for the procedural losses incurred were made The recovery of conjugates was not determined

TABLE 5. *Per cent recovery after paper chromatography of ³H NA and its metabolites.* The recovery of ³H NA and of its metabolites was determined by adding a known quantity of ³H-NA or metabolite to a foetal tissue homogenate. The recovery was determined after chromatography without hydrolysis (I n = 9–10) after acid hydrolysis for 15 min at 100 C in 0.4 mol/l perchloric acid (II n = 6–10) after sulphatase + β-glucuronidase hydrolysis for 24 hrs at 37°C (III n = 0–9) and after β-glucuronidase hydrolysis for 24 hrs at 37 C (IV n = 6–9)

Compound	I	II	III	IV
NA	94 1 ± 3.4	85.9 ± 3.3	86.0 ± 5 0	85.9 ± [illegible]
NMN	97 4 ± 2.0	88.6 ± 3.0	94.3 ± 1.3	95.2 ± 0.7
DHPG-DHMA	96.6 ± 3.0	89.4 ± 1 7	94 4 ± 1 7	93.5 ± 0.5
MHPG-VMA	94 4 ± 3.5	85.9 ± 4 4	91.6 ± 1 1	90.2 ± 2.2

Reproducibility of the paper chromatography procedure When seven replicate runs were made with the same foetal liver tissue homogenate, the percentage of the total activity found for the individual metabolite fractions was constant: the S.D of the different metabolite values was between 0.5 and 2 0 % of the total activity The mean values (in dpm) and coefficients of variation for the NA and metabolite fractions were: start, 57 dpm ± 20 4 %, conjugates, 155 dpm ± 4 9 %, NA, 58 dpm ± 12 1 %, NMN 34 dpm ± 23 8 % DHPG-DHMA 45 dpm ± 23 3 % MHPG-VMA, 420 dpm ± 6 1 % and front 22 dpm ± 16 4 % The coefficient of variation was highest for those metabolites which contained the lowest activity

Loss of activity during chromatography The aggregate activity (in dpm) of all the metabolites in a liver sample determined in seven replicate runs did not differ from the total values obtained without chromatography After chromatography 99.2 ± 4 2 % (mean ± S D) of the amounts determined without chromatography were obtained

Comment

In the paper chromatography procedure described above the separation of NA and its metabolites is very well defined. The conjugates have Rf values between 0 00 and 0 03 and are thus completely separated from NA. Only DHPG and DHMA on the one hand and MHPG and VMA on the other run close together However their separation is often of little significance in studies of NA kinetics. The method presented based on paper chromatography is rapid and simple. In addi

tion it is suitable for the analysis of large numbers of samples The large aliquot volume that can be applied to the paper is also a great advantage in that it allows lower activities than before to be measured reliably The recoveries of different metabolites are reasonably equal. A similar paper chromatography method (Rutschmann *et al.* 1965) had a greater number of steps and included lyophilizing steps which are very time-consuming The method employed by Kopin *et al.* (1961) is rather more complicated, and the recoveries of individual metabolites vary considerably The recovery values obtained with the present meth od compete well with those obtained with the cellulose plate chromatography method of Giese *et al* (1967)

RESULTS

I Rate of passage of ³H noradrenaline and its metabolites through the placenta

Material and methods

l-³H-NA, 2.8 μg or 100 μCi, was administered to seven mothers over a period of 10 seconds into the antecubital vein under general anaesthesia at the stage when the abdominal covers had been opened and the uterus was visible. The uterus was cut open 45 seconds after injection. Blood samples were taken simultaneously from the mother' peripheral blood, from the arm contralateral with the point of injection and from the umbilical vein and umbilical artery of the foetus 1 min, 2.5 min, 3.5 min and 5 min after injection. From one mother no blood samples were obtained and from one foetus only one sample. The blood that was drawn was pipetted into 0.4 mol/l perchloric acid at 0°C and cooled. The metabolites and total activity were determined as described in the section on methods.

Results

The results are shown in Tables 6–9 and in Figs 3–4 The ³H activity rapidly disappeared from the maternal blood and a measurable amount of H activity was already present in the umbilical blood of the foetus 1 min after the injection About 3.5 min after the injection an equilibrium appeared to exist between the radioactivity in the umbilical venous blood and the mother's peripheral blood. The ³H activity in the umbilical arterial blood 1–5 min after the injection was so low that it could not be ascertained with any certainty

The percentage of the total activity in the maternal blood present as ³H NA decreased rapidly 5 min after injection only about 25 of the ³H NA remained unmetabolized in the blood In the umbilical venous blood of the foetus the H NA level was very low it was mark

TABLE 6 H-NA and its metabolites in maternal peripheral blood and foetal umbilical venous blood 1 min after H-NA administration to the mother. During anaesthesia 2.8 μg or 100 μCi, of 1 H-NA were injected over 10 sec i to the mother' antecubital vein. Samples were taken from the arm contralateral from the injection, and simultaneously from the umbilical vei of the foetus. NA and metabolite quantities stated in Ci/g, metabolites in the umbilical blood also as percentage of the corresponding maternal metabolite (mean ± S.E.M) Number of deter minations in parentheses.

Compound	Maternal blood (5) nCi/g	Umbilical venous blood (6) nCi/g	(4) of corresponding maternal value
Start	0.64 ± 0.22	0.06 ± 0.03	10.6 ± 2.2
Conjugates	0 74 ± 0.11	0.02 ± 0.00[a]	3.5 ± 0.8
NA	4.22 ± 1.32	0.04 ± 0 00[a]	1.2 ± 0.2
NMN	0.27 ± 0.09	0.01 ± 0.00	5.6 ± 2.6
DHPG-DHMA	0.11 ± 0.01	0.04 ± 0 01	54.8 ± 25 6
MHPG-VMA	0.09 ± 0.01	0.12 ± 0.07	170.9 ± 106.3
Front	0.08 ± 0.03	0.05 ± 0.03	55.3 ± 44 0

p 0.05 difference versus maternal value

p 0.01

p 0.005

TABLE H-NA and its metabolites in maternal peripheral blood and foetal umbilical venous blood 2.3 min after H-NA administration to the mother. See legend to Table 6.

Compound	Maternal blood (5) nCi/g	Umbilical venous blood (6) nCi/g	(5) of corresponding maternal value
Start	0.36 0.06	0.14 ± 0.08	50.7 ± 20.7
Conjugates	0.37 ± 0.05	0.08 ± 0.03[a]	30.5 ± 10.7
NA	1.57 0.32	0.10 ± 0.02[a]	7.6 2.1
NMN	0 16 0.02	0.06 ± 0.02	56.0 ± 17 1
DHPG-DHMA	0.12 0.03	0.23 ± 0.07	447.8 ± 269.0
MHPG-VMA	0.13 0.02	0.83 ± 0.30[a]	861.6 ± 374 1
Front	0 05 0.02	0.06 ± 0.04	161.9 ± 67.9

p 0.01 – 0.05 difference versus maternal value

p 0.01

TABLE 8. H NA and its metabolites in maternal peripheral blood and fetal umbilical venous blood 35 min after H NA administration to the mother. See legend to Table 6.

Compound	Maternal blood (6) nCi/g	Umbilical venous blood (6) nCi/g	Umbilical venous blood (5) % of corresponding maternal value
Start	0.21 ± 0.04	0.15 ± 0.08	71.9 ± 24.8
Conjugates	0.27 ± 0.05	0.13 ± 0.02[b]	50.9 ± 8.5
NA	0.63 ± 0.17	0.12 ± 0.02[c]	19.1 ± 8.2
NMN	0.18 ± 0.02	0.09 ± 0.02	50.3 ± 9.4
DHPG-DHMA	0.11 ± 0.01	0.21 ± 0.05	291.7 ± 109.9
MHPG-VMA	0.18 ± 0.02	0.97 ± 0.39[a]	601.5 ± 155.4
Front	0.04 ± 0.01	0.07 ± 0.04	122.9 ± 51.3

$p = 0.1 - 0.05$ difference versus maternal value

$p < 0.05$

$p < 0.01$

TABLE 9. H NA and its metabolites in maternal peripheral blood and fetal umbilical venous blood 5 min after H NA administration to the mother. See legend to Table 6.

Compound	Maternal blood (6) nCi/g	Umbilical venous blood (5) Ci/g	Umbilical venous blood (4) % of corresponding maternal value
Start	0.24 0.06	0.09 ± 0.02	37.6 ± 14.4
Conjugates	0.30 ± 0.05	0.09 ± 0.02[a]	32.4 ± 10.9
NA	0.41 ± 0.07	0.09 ± 0.03[a]	23.6 ± 9.8
NMN	0.18 ± 0.03	0.08 ± 0.04	52.0 ± 22.0
DHPG-DHMA	0.14 ± 0.02	0.14 ± 0.05	163.7 ± 68.4
MHPG-VMA	0.20 ± 0.04	0.66 ± 0.28	637.3 ± 261.6
Front	0.06 ± 0.02	0.04 ± 0.01	194.7 ± 64.3

$p < 0.05$ difference versus maternal value

$p < 0.01$

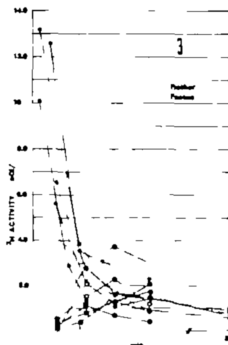


Fig. 3. *Total H concentration in maternal blood and foetal umbilical venous blood after administration of H NA to the mother* During anaesthesia, 2.8 μg, or 100 μCi of l-³H-NA was injected over 10 sec into the mother antecubital vein. Samples were taken from the mother's peripheral blood, and simultaneously from the umbilical vein of the foetus. Individual values are shown in the figure. From one mother no samples were obtained, and from one foetus only one sample. The results are given in nCi/g.

edly lower than that in the mother's peripheral blood ($p < 0.01$) and represented only about 10 % of the total at any time The amount present remained unchanged from 1 min onwards. However the proportion of the total activity in the umbilical venous blood present as ³H NA, relative to the amount in the mother's peripheral blood increased from 1 % to about 25 % as the ³H NA concentration in the maternal blood decreased

The per cent distribution of ³H NA metabolites in the foetal blood differed greatly from that observed in the maternal blood in the mother's blood the relative amounts of ³H NMN ³H DHPG DHMA and ³H MHPG-VMA increased uniformly with time, while in the foetal blood the level of ³H NMN remained constant at 5 % of the total activity and did not reach the level detected in the maternal blood The percentage from the total activity present as ³H DHPG DHMA and ³H MHPG VMA, combined was already 45 % 60 seconds after the injection and 65 – 75 subsequently The level of ³H MHPG-VMA was higher on a percentage basis, than in the maternal blood at all times. The amount of activity present in conjugated form was higher in the maternal than the foetal blood ($p < 0.05$ or $p < 0.005$)

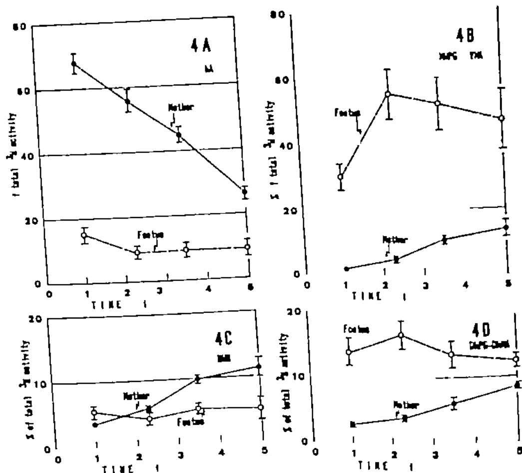


Fig. 4 *Presence of ³H NA and its metabolites in maternal blood and foetal umbilical venous blood 1–5 min after ³H NA administration to the mother.* Quantity of metabolites expressed as percentage of total activity (mean ± S.E.M.). — Fig. 4A: Quantity of NA, Fig. 4B: Quantity of MHPG-VMA, Fig. 4C: Quantity of NMN. Fig. 4D: Quantity of DHPG-DHMA. For other particulars, see legend to Fig. 3.

Comment

In the present study only some 10 % of the ³H activity was present in the umbilical vein of the foetus as ³H NA. Thus, little noradrenaline may pass, intact, through the placenta, like it was found by Sandler et al. (1963, 1964).

The results show that ³H NA is very rapidly metabolized as it traverses the placenta. For ³H MHPG-VMA production the ³H NA metabolism must be mediated both by COMT and MAO. Since the

centrations of ³H MHPG VMA were higher in the foetus than in the mother the metabolism obviously takes place in the placenta. The results were not very much affected by ³H NA metabolism in the foetus, as the activities in the umbilical artery were very low

Manipulation of the umbilical cord rapidly weakens the umbilical circulation as does the extraction of the foetus from the uterus (Rudolph *et al* 1971) It was obvious that foetal asphyxia developed during the procedure, but its strength was not measured Frequently however blood sampling after 5 min was already difficult, owing to closure of the umbilical blood vessels

II Distribution of ³H noradrenaline and its metabolites in foetal tissues after i v administration of ³H noradrenaline to the mother

Material and methods

1-³H NA, 1 2 μg or 50 μCi, was injected into the mother antecubital vein during anaesthesia. The samples were taken 5, 30 and 240 min after injection. In the case of those patients from whose foetus samples were taken after 5 min the injection was made t the stage when the abdominal covers including the peritoneum had been opened and the uterus exposed.

Those mothers whose foetus was sampled after 30 min recei ed the H NA injec tion before the commencement of the operation and those with a sampling time of 240 min, prior to premedication and operation.

After opening the uterus, the foetus was removed and blood samples were taken from the umbilical ein and arter and simultaneously from the mother s arm on the side contralateral with the point of injection. The foetal tissues were dissected in the following sequence: the heart was first removed, then the lungs, liver intestine ortic bodies, adrenals, kidneys, brain and brain stem and if possible, foetal urine was col lected. In a few nstances a sample of the mniotic fluid was taken before entering the uterus. The placental sample was taken last of all.

Both the 5-min and 30-min groups consisted of six subjects, nd the 240-min group of four The duration of pregnancy assessed by the size of the foetus, was from 11 to 0 weeks.

Results

Total activity

Blood. The ³H concentrations in the maternal blood and in the um bilical vein and artery of the foetus are shown in Fig 5 where the

means have been plotted and the vertical lines indicate the S.E.M. The ^{3}H activity disappears very rapidly from the maternal blood. The 1 min values were derived from mothers whose foetus was extracted 30 min after the injection. The individual values in two 1 min foetal blood samples are shown. Five, 30 and 240 min after injection the ^{3}H activity in the maternal blood and the umbilical vein blood was in equilibrium, and the levels were not significantly different.

The ^{3}H activity in the umbilical artery was significantly lower ($p < 0.005$) than that in the umbilical vein 5 min after injection, but after 30 and 240 min an equilibrium prevails between the ^{3}H concentrations in the umbilical vein and artery.

The rate of removal of the ^{3}H activity from the circulation during anaesthesia was not significantly different from that in the patients who received the injection prior to anaesthesia (Fig. 6).

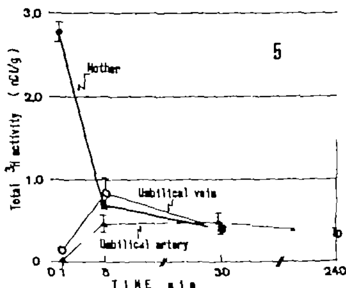


Fig. 5. *Total ^{3}H concentration in maternal blood and foetal umbilical venous and arterial blood after ^{3}H-NA administration to the mother.* During anaesthesia, 1.2 µg or 50 µCi of l-^{3}H-NA were injected over 10 sec into the mother's antecubital vein when samples were taken 5 or 30 min after the injection, and similarly prior to anaesthesia for sampling after 240 min. – ^{3}H activity is given in nCi/g. Only 2 foetal samples were obtained 1 min after the injection, other samples numbered between 4 and 10 per time interval, and the S.E.M is given in these cases.

Foetal tissues and placenta. The placenta contained a greater proportion of the ^3H activity than the foetal tissues (Tables 10—12) The placental activity was between 10 and 40 times the ^3H content of the liver The distribution of the total activity 5 and 30 min after the injection was for the most part similar the liver had received the greatest share, followed by the lungs hemispheres intestine and kidneys. The total activity in the aortic bodies was lowest. Four hours after injection the ^3H content of the lungs was highest, and the activity in the hemispheres had risen to about 80 % of that in the liver

The activity (in nCi/g) in the tissue is shown in Tables 10—12 The activity of the liver 5 min after the injection, was 0 88 ± 0 15 which is significantly higher than that in the placenta, lungs heart and brain and brain stem ($p < 0\,01$)

The activity in the liver 30 min after the injection, 2 10 ± 0 31 is significantly ($p < 0\,01$) higher than at 5 and 240 min The ^3H activity in the liver was higher than that in the heart lungs intestine, brain and brain stem ($p < 0\,05$—$0\,01$) The activity in the brain and

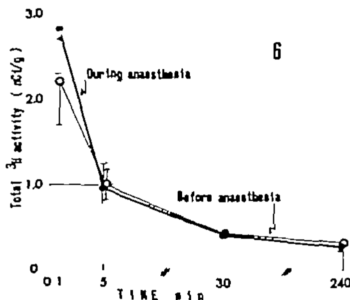


Fig 6. *Effect of anaesthesia on disappearance of H from the blood stream.* The figure is comparison between the rate of disappearance of H NA injected prior to anaesthesia and during anaesthesia. — Radioactivities in nCi/g mean ± S.E.M of 4—10 determinations. For other particulars, see legend to Fig 5.

brain stem was lowest on the average at all times, and there are no statistically significant differences in the 5 30 and 240-min values The ^3H activity in the placenta was also constant throughout the observation period. After 240 min, the ^3H activity in the aortic bodies was highest, 4 98 ± 0.33 while the activities in the other tissues were almost equal and close to their respective 5 min values. Comparison of the

TABLE 10. *Concentration and content of ^3H in placenta and foetal tissues 5 min after ^3H NA administration to the mother* Mean ± S.E.M of 5–6 determinations.

Tissue	^3H concentration nCi/g	% of ^3H concentration in the liver	Total ^3H content/tissue, nCi	% of ^3H content in the liver
liver	0.88 ± 0.15	100.0 ± 0.0	2.88 ± 0.85	100.0 ± 0.0
placenta	0.62 ± 0.16	69.9 ± 8.9	56.95 ± 13.28	2254.6 ±286.8
heart	0.41 ± 0.08	50.5 ± 11.3	0.23 ± 0.13	6.4 ± 1.5
lungs	0.24 ± 0.06	27.6 ± 8.9	1.26 ± 0.82	30.6 ± 10.1
intestine	0.53 ± 0.30	50.7 ± 17.5	1.23 ± 0.56	34.0 ± 8.9
aortic bodies	1.24 ± 0.84	154.9 ± 120.6	0.06 ± 0.05	1.2 ± 0.7
adrenals	0.52 ± 0.14	90.3 ± 47.8	0.27 ± 0 16	10.6 ± 5.6
kidneys	0.82 ± 0.07	116.1 ± 29.1	0.67 ± 0.25	26.8 ± 9.0
brain stem	0.08 ± 0.00	7.6 ± 1.8	0.11 ± 0.03	5.2 ± 1.3
hemispheres	0.12 ± 0.03	14.6 ± 5.8	1.87 ± 1 43	39.6 ± 18.6

TABLE 11 *Concentration and content of ^3H in placenta and foetal tissues 30 min after ^3H NA administration to the mother* Mean ± S.E.M of 5–6 determinations.

Tissue	^3H concentration, nCi/g	% of ^3H concentration in the liver	Total ^3H content/tissue, nCi	% of ^3H content in the liver
liver	2.10 ± 0.31	100.0 ± 0.0	4.51 ± 0.97	100.0 ± 0.0
placenta	0.59 ± 0.07	30.4 ± 5.4	42.46 ± 5 43	1079.9 ±146.6
heart	0.91 ± 0 14	44.8 ± 8.1	0.29 ± 0.09	6.5 ± 1 7
lungs	0.95 ± 0.08	54.5 ± 7.8	2.13 ± 0.86	40.2 ± 9.7
intestine	0.68 ± 0.14	34 1 ± 7.2	0.90 ± 0.30	20.1 ± 5.9
aortic bodies	3.80 ± 1.30	224.0 ± 86.4	0.16 ± 0.04	3.6 ± 1.0
adrenals	1.27 ± 0.22	63.9 ± 11.8	0.33 ± 0.11	6.9 ± 1 1
kidneys	1 42 ± 0.18	73.3 ± 11.2	0.68 ± 0.20	14.0 ± 2.3
brain stem	0.31 ± 0.04	16.1 ± 3.7	0.45 ± 0.09	11 7 ± 2.6
hemispheres	0.29 ± 0.06	13.7 ± 1 7	1.63 ± 0.40	33.5 ± 5.3

TABLE 12 *Concentration and content of H in placenta and foetal tissues 40 min after H N 1 administration to the mother* Mean ± S.E.M of 4 determinations.

Tissue	H concentration, nCi/g	% of H concentration in the liver	Total H content/tissue, nCi	% of ³H content in the liver
liver	0.50 ± 0.10	100.0 ± 0 0	1.27 ± 0.34	100.0 ± 0.0
placenta	0.59 ± 0.11	131.8 ± 65.0	56.57 ± 24.39	4040.3 ± 623.1
heart	0.87 ± 0.23	167 7 ± 21 0	0.27 ± 0.07	23.4 ± 6.0
lungs	0 60 ± 0.11	126.4 ± 16.2	1.67 ± 0 62	120.3 ± 15.1
intestine	0.37 ± 0.15	67.8 ± 13.3	0.52 ± 0.15	33.5 ± 10.2
aortic bodies	4.98 ± 0.35	1188 7 ± 292.4	0.21 ± 0.05	16.9 ± 4.3
adrenals	1.30 ± 0.31	254.0 ± 18.0	0.36 ± 0.06	30.0 ± 2.5
kidneys	0.62 ± 0.10	146.7 ± 34 0	0.50 ± 0.15	41 0 ± 7.8
brain stem	0.18 ± 0.06	35.0 ± 9.8	0.22 ± 0.07	20.4 ± 7 3
hemispheres	0.19 ± 0.00	39.9 ± 5.4	1.06 ± 0.31	79.6 ± 10.8

activity in the liver with the ³H activity in the umbilical vein of the foetus reveals that the ³H concentrations at 5 and 240 min (in nCi/g) did not differ significantly but that the activity in the liver 30 min after the injection, 2 10 ± 0 31 was significantly ($p < 0\ 001$) higher than the concentration in the umbilical vein and artery 0 42 ± 0 05 and 0 49 ± 0 11 respectively The ³H concentration in the kidneys, adrenals, heart lungs and aortic bodies was also higher than that in the umbilical vein, and that in kidneys and lungs higher than the concentration in the umbilical artery ($p < 0\ 01$) The ³H activities in the brain brain stem, intestine and placenta were not significantly different from the umbilical artery or vein values.

Metabolites

Blood 5 min after the injection the concentration of ³H NA in the mother's peripheral blood was significantly higher and that of ³H MHPG-VMA lower than in the umbilical vein of the foetus. The maternal ³H NA concentration was also significantly higher than in the umbilical artery (Table 13)

The ³H DHPG DHMA activity was higher in the umbilical vein of the foetus than in the maternal vein in five out of six foetuses. The fraction of metabolites travelling to the front was also higher ($p < 0\ 05$)

TABLE 13. ³H NA and its metabolites in maternal peripheral blood and foetal umbilical venous and arterial blood 5 min after ³H NA administration to the mother. During anaesthesia 1.2 µg or 50 µCi, of ³H NA were injected over 10 sec into the mother's antecubital vein. Samples were taken from maternal peripheral blood, contralateral from the injection, and simultaneously from foetal umbilical venous and arterial blood. NA and metabolite quantities stated in nCi/g (mean ± S.E.M, 6 determinations) and as average percentage of total activity

	Maternal blood (6)		Umbilical venous blood (6)		Umbilical arterial blood (6)	
Compound	nCi/g	% of total ³H activity	nCi/g	% of total ³H activity	nCi/g	% of total ³H activity
Start	0.09 ± 0.01	12.9	0.06 ± 0.02	6.7	0.05 ± 0.02	9.4
Conjugates	0.13 ± 0.02	18.6	0.07 ± 0.02³	7.8	0.05 ± 0.03³	9.4
NA	0.20 ± 0.03	28.6	0.08 ± 0.01	8.9	0.07 ± 0.02³	13.2
NMN	0.07 ± 0.01	10.0	0.08 ± 0.03	8.9	0.05 ± 0.01	9.4
DHPG-DHMA	0.08 ± 0.01	11.4	0.15 ± 0.05	16.7	0.10 ± 0.04	18.9
MHPG-VMA	0.12 ± 0.01	17.1	0.42 ± 0.10¹	46.7	0.17 ± 0.04	32.1
Front	0.01 ± 0.01	1.4	0.04 ± 0.04	4.4	0.04 ± 0.01	7.6

¹ $p < 0.02$ difference versus maternal value
² $p < 0.01$
³ $p < 0.005$

in the umbilical vein than in the maternal peripheral blood. The ³H MHPG-VMA concentration in the umbilical vein was higher than in the umbilical artery ($p < 0.02$)

The proportion of conjugated metabolites was higher in the maternal blood than in the umbilical vein or artery ($p < 0.005$). Five min after injection nearly 50 % of the activity behaved on chromatography like ³H MHPG-VMA, exactly as was found in study I

30 min after the injection the ³H MHPG-VMA concentration was still higher in the umbilical vein and artery than in the maternal peripheral blood ($p < 0.02$) (Table 14). 30 min after the injection the principal metabolite in the umbilical vein and artery was still ³H MHPG-VMA (accounting for about 1/4 of the activity)

The differences in the concentrations of the other metabolites were minimal with no statistically significant differences between the foetal and maternal blood.

After 240 min no statistically significant differences were evident between the concentrations of the various metabolites of ³H NA in the umbilical blood samples and in the maternal peripheral blood (Table 15)

TABLE 14 ³H NA *and its metabolites in maternal peripheral blood and foetal umbilical venous and arterial blood 30 min after ³H NA administration to the mother.* Samples taken 30 min after the injection. Number of determinations stated in parentheses. See legend to Table 13.

Compound	Maternal blood (5)		Umbilical venous blood (5)		Umbilical arterial blood (5)	
	nCi/g	% of total ³H activity	nCi/g	% of total ³H activity	nCi/g	% of total ³H activity
Start	0.10 ± 0.02	22.7	0.06 ± 0.01	14.3	0.09 ± 0.03	18.8
Conjugates	0.09 ± 0.02	20.5	0.08 ± 0.02	19.0	0.07 ± 0.02	14.6
NA	0.07 ± 0.02	15.9	0.05 ± 0.01	11.9	0.08 ± 0.02	16.7
NMN	0.03 ± 0.01	6.8	0.03 ± 0.00	7.1	0.03 ± 0.01	6.3
DHPG-DHMA	0.05 ± 0.01	11.4	0.06 ± 0.01	14.3	0.05 ± 0.01	10.4
MHPG-VMA	0.07 ± 0.01	15.9	0.11 ± 0.01	26.2	0.13 ± 0.03[1]	27.1
Front	0.03 ± 0.01	6.8	0.03 ± 0.01	7.1	0.03 ± 0.01	6.3

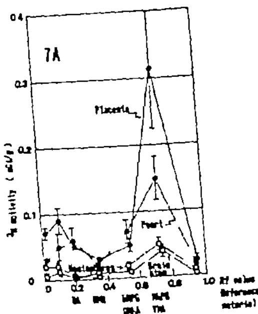
[1] $p < 0.02$ difference versus maternal value

TABLE 15. ³H NA *and its metabolites in maternal peripheral blood and foetal umbilical venous and arterial blood 240 min after ³H-NA administration to the mother.* Prior to anaesthesia, ³H NA was injected into the mother's antecubital vein. Samples were taken 240 min after the injection. Number of determinations in parentheses. See legend to Table 13.

Compound	Maternal blood (4)		Umbilical venous blood (4)		Umbilical arterial blood (4)	
	nCi/g	% of total ³H activity	nCi/g	% of total ³H activity	nCi/g	% of total ³H activity
Start	0.08 ± 0.01	24.2	0.07 ± 0.01	21.2	0.08 ± 0.03	24.2
Conjugates	0.07 ± 0.02	21.2	0.06 ± 0.01	18.2	0.06 ± 0.01	18.2
NA	0.04 ± 0.00	12.1	0.04 ± 0.02	12.1	0.04 ± 0.01	12.1
NMN	0.02 ± 0.00	6.1	0.02 ± 0.01	6.1	0.02 ± 0.01	6.1
DHPG-DHMA	0.03 ± 0.00	9.1	0.04 ± 0.02	12.1	0.04 ± 0.01	12.1
MHPG-VMA	0.05 ± 0.02	15.2	0.07 ± 0.01	21.2	0.07 ± 0.02	21.2
Front	0.04 ± 0.02	12.1	0.03 ± 0.00	9.1	0.02 ± 0.01	6.1

Foetal tissues and placenta. As in the blood ³H MHPG-VMA was the most abundant metabolite on a percentage basis in all foetal tissues except the lungs kidneys and adrenals 5 min after the injection. The dominant component in the kidneys and adrenals were metabolites which behaved chromatographically as conjugates (Figs 7—8, Table 16)

However 30 min after injection metabolites behaving as conjugates were also present in high amounts (Figs 8—9 Table 17—18)



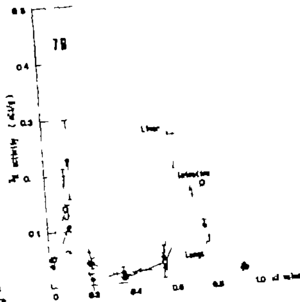
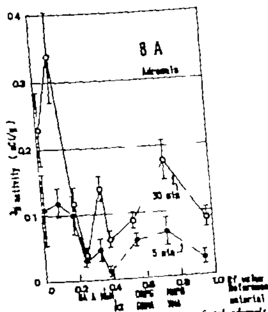


Fig. 7 ³H-NA and its metabolites in foetal tissues 5 min after ³H-NA administration to the mother. Quantity of metabolites in nCi/g. Mean ± S.E.M. values of 3 to 6 foetuses shown in the figure. — Fig. 7A. Beha iour of reference substances on chromatography. See legend to Fig. 3.



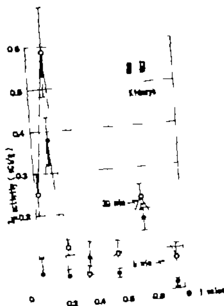


Fig. 8. ³H-NA and its metabolites in foetal adrenals and kidneys 5 and 30 min after ³H-NA administration to the mother. Quantity of metabolites in nCi/g. Mean ± S.E.M. values for 6 foetuses. Fig. 8A: Distribution of metabol tes in adrenals. Fig. 8B: Distribution of metabolites in kidneys. The behaviour of reference material is shown in Fig. 8A. See legend to Fig. 3.

TABLE 16. *Determination of unconjugated and conjugated ³H NA and its metabolites in the foetal liver 5 min after ³H NA administration to the mother* Samples taken 5 min after injection of 1 2 μg or 50 μCi of ³H NA into the mother' antecubital vein. Metabolites determined by paper chromatography without hydrolysis and after acid hydrolysis by heating in 0 4 mol/l perchloric acid in boiling water for 12—15 min. Quantities of NA and metabolites in nCi/g (mean ± S.E.M) and average percentages of total activity Number of determinations in parentheses.

Compound	Without hydrolysis (5) nCi/g	Without hydrolysis (5) % of total ³H activity	After acid hydrolysis (6) nCi/g	After acid hydrolysis (6) % of total ³H activity
Start	0.05 ± 0.01	5.7	0.03 ± 0.00	3.4
Conjugates	0.23 ± 0.07	26.4	0.06 ± 0.01	6.7
NA	0.04 ± 0.01	4 6	0 19 ± 0.04	21.5
NMN	0.01 ± 0.00	1 1	0.11 ± 0.01	12.4
DHPG-DHMA	0.04 ± 0.00	4.6	0.04 ± 0.01	4.5
MHPG-VMA	0.49 ± 0.11	56.3	0.44 ± 0.09	49 4
Front	0.01 ± 0.00	1 1	0 02 ± 0.00	2.2

$p < 0.02$ difference versus value without hydrolysis
p 0.01
p 0.005

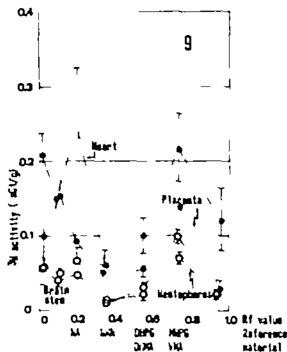


Fig. 9 *³H NA and its metabolites in foetal tissues 30 min after ³H NA administration to the mother* 1.2 μg or 50 μCi, of ³H NA injected over 10 sec into the mother' antecubital vein. Foetal tissue samples taken 30 min after the injection. NA and metabolite quantities in nCi/g mean ± S.E.M for 5 to 6 foetuses.

TABLE 17. *Unconjugated and conjugated H VI and its metabolites in foetal lung and intestine 30 min after H VI administration to the mother.* Samples taken 30 min after the injection. See legend to Table 16.

	Lungs (5)				Intestine (4)			
	Without hydrolysis		After acid hydrolysis		Without hydrolysis		After acid hydrolysis	
Compound	nCi/g	% of total H activity	nCi/g	of total ³H activity	nCi/g	% of total H activity	nCi/g	of total H activity
Start	0.24 ± 0.06	25.5	0.03 ± 0.01	3.2	0.11 ± 0.03	21.2	0.04 ± 0.01	7.5
Conjugates	0.45 ± 0.04	47.9	0.07 ± 0.01	7.4	0.14 ± 0.05	26.9	0.04 ± 0.01	7.5
NA	0.06 ± 0.01	6.4	0.10 ± 0.03	10.5	0.07 ± 0.02	13.5	0.07 ± 0.01	13.2
NMN	0.02 ± 0.01	2.1	0.05 ± 0.01	5.3	0.02 ± 0.00	3.8	0.05 ± 0.01	9.4
DHPG-DHMA	0.03 ± 0.01	3.2	0.06 ± 0.01	6.3	0.03 ± 0.01	5.8	0.05 ± 0.01	9.4
MHPG-VMA	0.11 ± 0.01	11.7	0.54 ± 0.04	56.8	0.12 ± 0.02	23.1	0.21 ± 0.05	39.6
Front	0.03 ± 0.01	3.2	0.10 ± 0.02	10.5	0.03 ± 0.01	5.8	0.07 ± 0.07	13.2

$p < 0.05$ difference versus value without hydrolysis
$p < 0.01$
$p < 0.005$

Table 18. *Determination of conjugated ³H NA metabolites in foetal liver 30 min after ³H-NA administration to the mother* Samples taken 30 min after injection of 1.2 μg, or 50 μCi, of ³H-NA into the mother's antecubital vein. Metabolites determined before hydrolysis and after both acid and enzyme hydrolyses. Acid hydrolysis was by heating for 12–15 min in 0.4 mol/l perchloric acid in boiling water Enzyme hydrolysis was with a sulphatase + β-glucuronidase preparation (Glusulase®) for 24 hrs at 37°C. Quantity of metabolites stated i nCi/g (mean ± S.E.M) and a erage percentage of total activity Number of determinations in parentheses.

	Without hydrolysis (5)		After acid hydrolysis (5)		After sulphatase + β-glucuronidase hydrolysis (5)	
Compound	nCi/g	of total H activity	nCi/g	% of total H activity	nCi/g	% of total H activity
Start	0.29 ± 0.05	15.9	0.03 ± 0.01	1 7	0.04 ± 0 01	2.2
Conjugates	0.31 ± 0.03	17 0	0.06 ± 0.02[a]	3.3	0.07 ± 0.02[a]	3.8
NA	0.09 ± 0.01	4.9	0.09 ± 0.02	5.0	0.03 ± 0.01	1.6
NMN	0.05 ± 0.01	2.7	0.33 ± 0.03[c]	18.3	0.32 ± 0.04[a]	17.6
DHPG-DHMA	0.08 ± 0.01	4 4	0.04 ± 0.01	2.2	0.02 ± 0.01	1 1
MHPG-VMA	0.93 ± 0.08	51 1	1 17 ± 0.08[a]	65.0	1.29 ± 0.06[c]	70.9
Front	0.07 ± 0.02	3.8	0.08 ± 0.02	4 4	0.05 ± 0.03	2.7

$p < 0.05$ difference versus value without hydrolysis
$p < 0.01$
p 0.001

The sample quantities from the aortic bodies were so small that the metabolites could only be analysed in a part of them. In these cases *descending chromatography* was used The *separation* of *NA/A* and NMN/MN was fairly good though not as complete as that of the different metabolites of NA. It is observed that ³H A and ³H MN were not positively demonstrable in the aortic bodies or in the adrenals.

240 min after the injection a large proportion of the metabolites behaved as conjugates. In this connection only the distribution of metabolites from the heart, liver intestine and brain stem are presented (Fig 10)

In the case of the 5 min samples, acid hydrolysis and separation of conjugated metabolites was only carried out with liver lung and intestinal homogenates, and only if there was sufficient material for several runs (Table 16)

All these determinations yielded similar results 5 min after the injection ^{3}H NA and ^{3}H NMN could be found in the foetus in conjugated form.

30 min after the injection, ^{3}H NMN and part of the ^{3}H MHPG VMA were also present in the same tissues in conjugated form. This was established using both acid hydrolysis and enzymic hydrolysis with Glusulase® which contains both sulphatase and β-glucuronidase (Tables 17–18)

The umbilical blood samples and the samples from the foetal adrenals, kidneys and paraganglia were not large enough for conjugate determinations. Conjugates were not analysed in the samples taken 210 min after the injection. The quantities of amniotic fluid and urine collected and the ^{3}H activity in them were too small to permit any statement concerning their metabolite content.

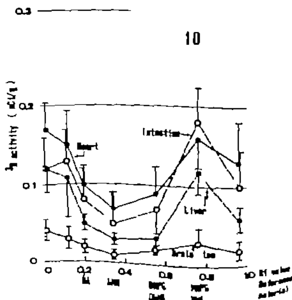


Fig. 10 *H-NA and its metabolites in foetal tissues 240 min after H NA administration to the mother* Foetal tissue samples taken 240 min after the injection. NA and metabolite quantities (nCi/g mean ± S.E.M of 4 foetuses For other particulars, see legend to Fig. 9

Comment

All the ^{3}H NA metabolites were found in the foetal umbilical blood 5 30 and 240 min after injection. The ^{3}H MHPG-VMA level was higher in the foetal than in the maternal blood therefore ^{3}H NA is metabolized in the placenta and/or ^{3}H MHPG VMA cannot pass freely from the foetus to the mother

^{3}H NA metabolites accounted for the major part of the activity in the foetal tissues. In view of the minimal amount of intact ^{3}H NA that can pass from the mother into the foetus, the ^{3}H NA uptake capacity of the foetal tissues was not determined in these experiments.

The placenta metabolizes ^{3}H NA, and the foetus obviously continues this metabolic action with the aid of both MAO and COMT The foetus also has capacity to conjugate these metabolites. In the placenta no p-nitrophenol-sulphating activity (Pulkkinen 1963) nor any activity of the enzymes participating in the glucuronic acid conjugation of o-aminophenol (Hartiala and Pulkkinen 1955 Dutton 1959) could be detected It is therefore probable that no conjugation of catecholamines occurs in the placenta. However the present study does not exclude the possibility that metabolites conjugated in the mother might pass into the foetus

III Metabolism of ^{3}H noradrenaline in the foetoplacental unit

Material and methods

In this part of the study a total of 13 foetuses weighing from 15 to 274 g were investigated dl-^{3}H NA 25–85 μCi/kg or 0 6 to 2.1 μg/kg was injected into the umbilical vein of eight foetuses (20 274 g) over a period of 30 seconds. Efforts were made to keep the dose as near to 50 μCi/kg as possible In order to estimate its weight the foetus was removed from the uterus in every case dl-^{3}H NA was injected into the jugular vein of three other foetuses after dissection and at later stage ^{3}H NA was injected into the jugular vein of two further foetuses, 53 and 24 μCi/kg or 1 0 and 0.45 μg kg respectively The foetoplacental circulation was maintained intact for 15 min. The umbilical cord was then closed and the foetus and placenta placed in separate containers. One hour after the start of the injection, the tissues were excised in the same order as in the preceding studies: heart lungs, liver intestine adrenals, kidneys, brain and brain stem

The metabolites were determined in a part of the tissues, only the weighed tissue was placed in 0.4 mol l perchloric acid, continuing thereafter as stated in the chapter on methods. Part of the tissues were dried at –4 C and their total activity determined by combustion.

The mother's premedication regimen consisted of the following: atropine 0.1 mg/10 kg, pethidine 50—100 mg and prometazine 25 mg i.m. one hour before the operation. Induction was carried out with thiomebumal, and the anaesthesia was continued with a mixture of N_2O/O_2 and, for relaxation of the uterus, halothane at a concentration of 0.5 % until the foetus and placenta had been detached and the umbilical circulation closed.

The metabolites were determined in the same manner as in the preceding studies, except that the whole of the activity running below NA was determined as one single strip and that running above VMA was not determined.

Results

Total activity

The activity in the tissues, in nCi/g of tissue, and their content per tissue are shown in Table 19. It can be seen that the ^{3}H activity is remarkably uniformly distributed between the different tissues, and that the mode of injection had some influence on the distribution. The proportion taken up by the liver decreased ($p < 0.01$) and that by the placenta increased when the injection was made into the jugular vein. The ^{3}H activity (in nCi/g) in the brain and brain stem was very similar but lower on the average than in the other tissues ($p < 0.001—0.01$). No statistically significant differences were apparent between any of the other tissues.

The ^{3}H content of the placenta (nCi) was highest, followed by the liver and the lungs.

All data showed a considerable dispersion, and the dispersion was also rather high in the amount of ^{3}H activity injected. The ^{3}H activity in the tissues examined amounted to 10—15 % of the quantity injected. Blood samples were drawn from three mothers 15 min after the injection, and it could be seen that some activity passes from the foetus into the mother. However, the amount was so small (0.08 nCi/ml) that metabolite determination was not possible.

The foetuses injected with l-^{3}H NA were examined at a later time, in 1972, and are therefore considered separately. On account of the small subject number no definite conclusions can be drawn, but it appears possible that the l-^{3}H NA uptake by the foetal tissues is higher than that of the dl-form, or that it is metabolized to a lesser degree (Table 20).

The activity in the aortic bodies was highest, and that in the brain lowest. Otherwise the distribution was fairly uniform.

TABLE 19 *Distribution of H activity in placenta and foetal tissues after administration of ³H-NA into umbilical or jugular vein of foetus in the fetoplacental unit.* Mean ± S.E.M of 3 (administration to jugular vein) to 7–8 determinations (administration to umbilical vein)

Tissue	H concentration, nCi/g	% of ³H concentration in the liver	Total ³H content/tissue nCi	% of H content in the liver
³H-NA administration into the umbilical vein				
liver	25.8 ± 4.4	100.0 ± 0.0	108.7 ± 39.7	100.0 ± 0.0
placenta	1.5 ± 0.4	7.6 ± 2.3	165.7 ± 51.2	299.0 ± 138.0
heart	13.7 ± 0.4	72.3 ± 17.1	8.2 ± 2.7	11.3 ± 3.0
lungs	18.1 ± 3.4	89.1 ± 25.2	63.8 ± 27.9	115.4 ± 36.1
intestine	10.8 ± 2.7	74.4 ± 30.5	20.3 ± 4.0	68.7 ± 24.3
adrenals	22.8 ± 5.0	97.5 ± 23.1	10.1 ± 3.7	12.2 ± 3.3
kidneys	14.3 ± 3.8	68.7 ± 15.8	16.3 ± 8.6	16.2 ± 5.1
brain stem	2.8 ± 0.7	14.7 ± 5.9	3.8 ± 1.0	9.0 ± 4.2
hemispheres	2.9 ± 0.8	16.2 ± 6.4	27.9 ± 11.5	54.0 ± 18.6
H-NA administration into the jugular vein				
liver	4.4 ± 1.0	100.0 ± 0.0	11.9 ± 5.3	100.0 ± 0.0
placenta	7.4 ± 3.1	148.4 ± 16.5	747.9 ± 384.9	4222.8 ± 1621.3
heart	18.2 ± 8.2	507.8 ± 239.7	4.6 ± 2.1	67.3 ± 32.8
lungs	5.0 ± 1.0	107.8 ± 13.8	17.7 ± 5.0	107.6 ± 0.0
intestine	7.0 ± 0.2	174.7 ± 37.5	10.7 ± 4.0	93.0 ± 17.2
adrenals	18.9 ± 12.5	513.7 ± 348.2	2.3 ± 0.5	44.6 ± 31.4
kidneys	16.3 ± 6.8	417.7 ± 203.2	5.4 ± 1.3	65.4 ± 26.0
brain stem	1.4 ± 0.0	33.2 ± 12.5	2.5 ± 0.1	16.3 ± 4.9
hemispheres	1.9 ± 1.3	52.1 ± 42.4	16.4 ± 10.6	124.6 ± 92.4

TABLE 20 *Concentration and content of ³H in placenta and foetal tissues after administration of ³H-NA into jugular vein of foetus in the fetoplacental unit.* Individual values of two foetuses; weight of foetuses in parentheses.

Tissue	H concentration, nCi/g		Total H content/tissue nCi	
	(94 g)	(253 g)	(94 g)	(253 g)
liver	43.3	21.0	160.8	221.7
placenta	0.8	8.0	93.2	1051.9
heart	129.9	92.0	57.2	135.1
lungs	137.6	25.3	590.1	173.1
intestine	32.7	23.6	68.3	116..
aortic bodies	324.8	158.9	8.1	7.8
adrenals	167	31.5	56.2	20.1
kidney	41.1	51.0	42.6	91.4
brain stem	4.6	1.1	8.5	2.6
hemispheres	2.4	1.0	4.3	31.6

Since the ³H NA dosage per unit weight was highly variable, the metabolite levels are considered on the basis of their relative (percentage) distribution. dl ³H NA accounted for the greatest part of the activity in the heart, intestine, kidneys and adrenals, on the average 40—55 % of the total (Figs 11—12) In the adrenal samples NA and A were

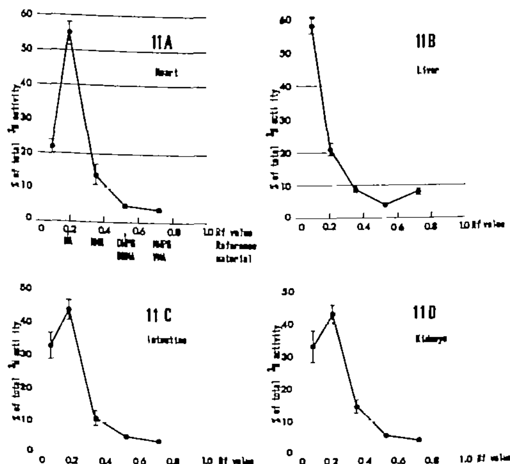


Fig. 11 *Metabolism of H NA in the foetoplacental unit.* 0.6 to 2.1 μg/kg of dl-³H-NA injected into the foetoplacental unit through the umbilical vein or jugular vein of the foetus. Umbilical cord clamped after 15 min. Metabolite quantities given as percentage of total activity The results are mean ± S.E.M values for 6 to 8 foetuses. — Fig. 11A. Foetal heart, Fig 11B: Foetal liver Fig. 11C: Foetal intestine, Fig. 11D: Foetal kidneys. Fig. 11A shows the behaviour of the reference substances on chromatography

not separated In the remainder of the tissues the major part of the activity behaved on chromatography as conjugated more than 55 % in the liver and on average, 40 % in the lungs. The proportion of conjugate like activity was lowest in the heart, about 22 % and in the other tissues about 30—35 %

A very similar per cent distribution was observed in the two foetuses injected with l ³H NA. However the proportion of ³H NA in the heart intestine and kidneys was higher than in the foetuses which were injected with dl ³H NA (Figs 13—15) The l ³H NA metabolite distribution in the blood and brain of these foetuses were very similar Approximately half of the substance administered behaved as NA on chromatography

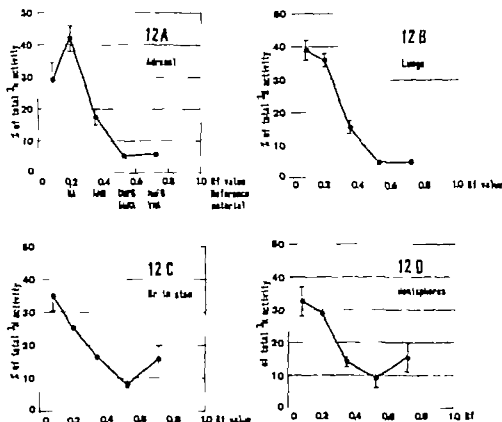
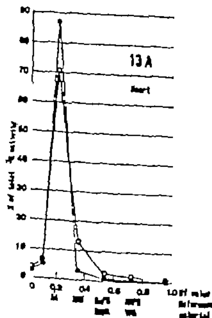
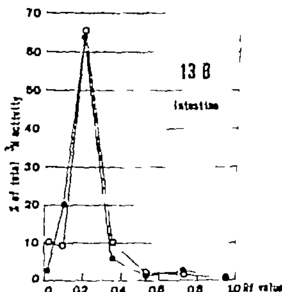


Fig. 12. *Metabolism of ³H NA in the foetoplacental unit.* Fig. 12A: Metabolites in the f[oetal] adrenals, Fig. 12B: in foetal lungs, Fig. 12C: in foetal brain stem, Fig. 12D: in foetal [hemis]pheres. Mean ± S.E.M. values for 6 to 8 foetuses. For other particulars see legend to F[ig.]





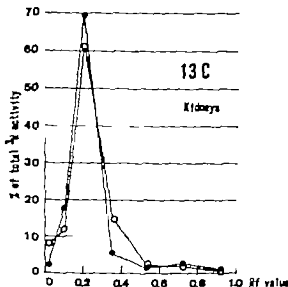


Fig. 13. *Metabolism of ³H-NA in the foetoplacental unit.* 0.45 μg/kg of ³H-NA administered to one foetus, and 1.0 μg/kg to another by injection into the jugular vein. Umbilical cord blocked after 15 min. Results stated as percentages of total activity. — Fig. 13A: Metabolites in foetal heart, Fig. 13B: in foetal intestine, Fig. 13C: in foetal kidneys. Fig. 13A shows the behaviour of reference material on chromatography. ○ Foetus weighing 233 g. ● Foetus weighing 94 g.

Of the metabolites of dl ^{3}H NA and l ^{3}H NA, more NMN than DHPG-DHMA was found, and usually equal amounts of MHPG-VMA and DHPG DHMA. In the paraganglia and adrenals of two foetuses no l ^{3}H A or l ^{3}H MN could be detected (Fig 15) The conjugates were also determined, after acid hydrolysis and enzymic hydrolysis, in the liver supernatant of the foetuses injected with l ^{3}H NA. It could be clearly seen that in both foetuses ^{3}H NA and ^{3}H NMN were present in the liver conjugated to sulphate (Fig 16)

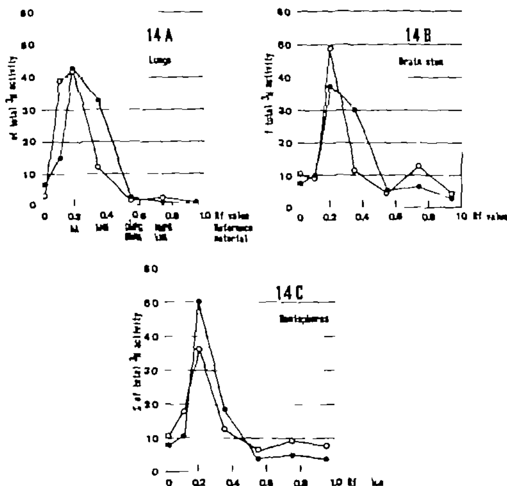
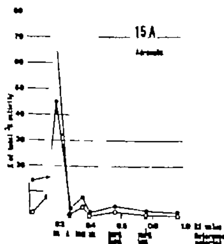


Fig. 14 *Metabolism of H N I in the foetoplacental unit.* Fig. 14 A. Metabolites in foetal lungs, Fig. 14B. in foetal brain stem Fig 14C. foetal hemispheres. Fig. 14 A shows the behaviour of reference compounds in chromatography For other particulars, see legend to Fig. 13.



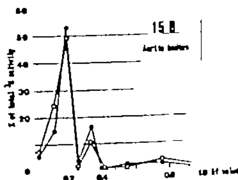


Fig. 15. *Metabolism of H-NA in the foetoplacental unit* Fig. 15A: Metabolism adrenals, Fig. 15B: Metabolism in aortic bodies. For other particulars, see legend to Fig 13.

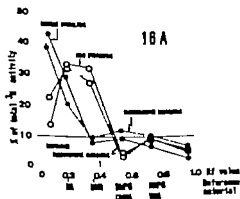




Fig. 16. *Conjugates of H-NA and its metabolites in the foetal liver in the foetoplacental unit* I the foetoplacental unit I ³H-NA was injected into the jugular el of two foetuses, 0.45 µg/kg into one and 1.0 µg/kg into the other The umbilical cord was blocked after 15 min. Metabolites determined before hydrolysis and after acid as well as enzymic hydrolysis. Acid hydrolysis was performed by heating the supernatant, prior to chromatography for 12 min in boiling water in 0.4 mol/l perchloric acid, enzyme hydrolysis using an enzyme preparation containing sulphatase and β-glucuronidase (Glusulase®) or preparation containing β-glucuronidase (Ketodase®) for 24 hrs at 37°C. Metabolic quantities as percentages of total activity Fig. 16A: Foetus weighing 94 g. Fig. 16B: Foetus weighing 253 g.

Comment

The experimental design resulted in foetal asphyxia the degree of which was not measured However the heart functioned in all foetuses after 15 min It is clear from this study that the foetus is well able to metabolize ^{3}H NA MAO COMT and sulphate conjugating activity was detected

The heart, intestine, kidneys and adrenals of the foetus can obviously actively remove ^{3}H NA from the circulating blood judging from the remarkable proportion of ^{3}H NA in them. nearly 90 % in the heart of one foetus injected with 1 ^{3}H NA. The brain contained a distinctly measurable quantity of ^{3}H although the amount of ^{3}H NA was only about 1/3 of the total The blood/brain barrier is clearly not yet fully developed in a foetus of this age. The tissue concentrations in the foetuses injected with 1 ^{3}H NA were fairly high which is partly due to the fact that the material was injected into the jugular vein the dissection of which is rather time-consuming As a consequence, the placental circulation has time to deteriorate, and the leakage of ^{3}H activity into the mother becomes less

IV Distribution and metabolism of ^{3}H noradrenaline in the human foetus after removal from the uterus

Material and methods

The material for this study comprised six foetuses aged 13 to 20 weeks weighing between 35 and 260 g The foetuses were obtained at the interruption of pregnancy performed by the laparomoty approach

After removal of the foetus and placenta from the uterus, the umbilical cord was closed within 1 min and 1 H NA (specific activity 8.7 Ci/mmol) 0.11 ± 0 01 μCi/100 g or ca. 0.02 μg/kg was injected over a period of 10 seconds into the umbilical tump In view of the olume error the umbilical cord was not flushed, instead it was "milked" towards the foetus. The foetus was transferred within 2 min from the operating theatre in a covered cardboard box, to a heating bath at 37 C, where it was left for 15 min. During and all through the last minut the foetal heart rate was counted. A blood sample was then taken by making an opening in the right atrial wall of the heart with a scissors and drawing the blood from the cardiac sac with a pipette This procedure took 1 – 2 minutes. The blood was directly pipetted into 0 4 mol/l perchloric acid, at 0 C on an ice bath. The heart then was removed A piece of the liver was also immersed, at this time in 0 4 mol/l perchloric acid, and rapidly cooled to – 15 – – 20 C. The other tissues were excised, weighed and dried for a few days at +4 C. The metabolites and conjugates in the blood liver and urine of the foetus were analysed using the method presented in the chapter on methods. In the rest of the tissues the tissue activity was determined by combustion of the total organ or part thereof.

Results

After 15 min, the heart rate of the foetus averaged 75 ± 8 beats per min (mean ± S.D.) In one foetus the following Astrup values were obtained. pH 6.94 pCO_2 78.6 mmHg and BE −15.6 meq/l This particular foetus had a heart rate of 62 beats per min during the last minute.

Total activity

The ^3H activity in the tissues is given in Table 21 The activity in the aortic bodies, averaging 8 18 ± 0.59 nCi/g, was higher than in any of the other foetal tissues ($p < 0.001$)

There were no statistically significant differences in the total activity in the heart, liver and adrenals. The activity in the liver 3 93 nCi/g, was higher than that in the lungs ($p < 0.005$) intestine ($p < 0.005$) kidneys and brain and brain stem. The activity in the brain and brain stem (0 15 and 0 16 nCi/g) was significantly lower than in all other tissues, including the skin of the head ($0.01 > p < 0.001$)

The activity in the blood urine and skin was almost the same, 1 08 ± 0 17 0 96 ± 0.28 and 0 96 ± 0 08 nCi/g, respectively ($p > 0.05$)

The total ^3H content, calculated per tissue, was significantly greater in the liver ($p < 0.001$) than in all the other tissues. Of the remainder

TABLE 21 *Concentration and content of ^3H in foetal tissues after administration of ^3H-NA into umbilical vein after clamping umbilical cord.* Mean ± S.E.M of 6 determinations.

Tissue	^3H concentration, nCi/g	% of ^3H concentration in the liver	Total ^3H content/tissue, nCi	% of ^3H content in the liver
liver	3.93 ± 0.49	100.0 ± 0.0	21.99 ± 7 71	100.0 ± 0.0
heart	3.44 ± 0.41	98.2 ± 19.9	2.28 ± 0.80	10.5 ± 1.8
lungs	1 14 ± 0.19	31.5 ± 5.8	7 01 ± 2.71	27.5 ± 5.5
intestine	1.38 ± 0.28	35.7 ± 5.3	4.77 ± 1.80	22.1 ± 3.8
aortic bodies	8.18 ± 0.59	224.5 ± 29.5	0.12 ± 0.01	1.3 ± 0.4
adrenals	2.77 ± 0.28	78.0 ± 16.9	1.33 ± 0.36	6.4 ± 0.6
kidneys	1.89 ± 0.32	40.4 ± 6.1	1.85 ± 0.42	10.2 ± 2.0
brain stem	0.15 ± 0.00	4.2 ± 0 7	0.35 ± 0.10	1.8 ± 0.4
hemispheres	0.16 ± 0.00	4.5 ± 1.0	2.53 ± 0.87	11 7 ± 1 9
skin of the head	0.96 ± 0.08	26.9 ± 4.3		—

the lungs and intestine contained the highest activity The heart and brain contained almost equal amounts. 2 28 $\pm$ 0 80 and 2.53 $\pm$ 0 87 nCi, respectively The activity was lowest in the para aortic ganglia, 0 12 $\pm$ 0 01 nCi (Table 21)

The activity contained in the tissues taken as samples accounted (on the average) for 27 7 $\pm$ 2 9 % of the radioactive substance injected. This value is quite consistent with the proportion of the total foetal weight in the samples analyzed which was 25 3 %

Metabolites

If the average metabolite distribution at a given time in the liver blood and urine (Table 22) are compared, it can be seen that the metabolite pattern in the blood and urine is almost identical. On the basis of this evidence, under these experimental conditions about half of the ^{3}H NA metabolites would be excreted in unchanged form in the urine and the end product MHPG-VMA would only account for about 7 % of the total. On the other hand the proportion of conjugated metabolites in the liver was much higher than in the blood and urine. The same is true of the ^{3}H DHPG-DHMA and ^{3}H MHPG-VMA concentrations (0 01 $> p <$ 0 001) It was clear from the results that the foetus can metabolize injected NA independent of the placenta, through the action of both MAO and COMT

TABLE 22. *Fate of ³H-NA in the foetus outside the uterus.* 0.02 µg/kg or 1 1 µCi/kg, of 1 ³H NA was injected into the umbilical vein of the human foetus after clamping the umbilical cord. Samples were taken 15 min after the injection. Quantity of NA and metabolites in nCi/g (mean $\pm$ S.E.M, 4–6 determinations) and average percentages of metabolites from total activity Number of determinations in parentheses

	Liver (6)		Blood (6)		Urine (4)	
Compound	nCi/g	% of total ³H activity	Ci/g	% of total ³H activity	nCi/g	% of total ³H activity
Start	0.56 $\pm$ 0.14	14.9	0.06 $\pm$ 0.01	5.6	0.12 $\pm$ 0.07	13.2
Conjugates	1.55 0.32	41.2	0.17 $\pm$ 0.03	15.7	0.09 $\pm$ 0.04[1]	9.9
NA	0.74 0.08	19 7	0 44 $\pm$ 0 11	40.7	0.46 $\pm$ 0.18	50.5
NMN	0.23 0.02	6.1	0 14 $\pm$ 0.03	13.0	0.07 $\pm$ 0.02	7 7
DHPG-DHMA	0.22 0.03	5.9	0.10 $\pm$ 0.02	9.3	0.07 $\pm$ 0.02	7 7
MHPG-VMA	0.38 0.05	10.1	0.09 $\pm$ 0.01[1]	8.3	0.06 $\pm$ 0.02[1]	6.6
Front	0.08 0.02	2.1	0.08 $\pm$ 0.02	7 4	0.04 $\pm$ 0.01	4 4

$p < 0.01$ difference from value in the liver
$p < 0.005$

The conjugated metabolites were analysed both after acid and enzymic hydrolysis, in order to determine the mode of conjugation

The chromatogram obtained without preceding hydrolysis is clearly different from those after acid and Glusulase* hydrolysis (Table 23) The activity in the conjugate region was reduced by acid hydrolysis ($p < 0.01$) the proportion of NA correspondingly increased from 0 74 ± 0.08 to 1 23 ± 0 16 ($p < 0\ 02$) and that of NMN from 0 23 ± 0 02 to 1.28 ± 0.24 nCi/g ($p < 0\ 01$) The fraction running above VMA increased from 0.08 ± 0 02 to 0 16 ± 0 03 ($p < 0\ 02$)

After treatment with both β-glucuronidase and sulphatase the fraction remaining at the start did not differ significantly from the unhydrolysed value. On the other hand the fraction running up over the start, 0.33 ± 0.05 nCi/g, was significantly ($p < 0\ 01$) lower than in the absence of hydrolysis. The NA ($p < 0\ 02$) and NMN ($p < 0\ 01$) levels were also significantly increased.

β-glucuronidase treatment did not alter the percentage distribution of the metabolites. Only the activity in the fraction running ahead of VMA was seen to increase from 0 08 ± 0 02 to 0.26 ± 0 04 nCi/g ($p < 0.01$)

TABLE 23. *Conjugation of H NA and its metabolite in the foetal liver outside the uterus* Metabol tes determined before hydrolysis and after acid and enzyme hydrolysis. Acid hydrolysis w by heating for 12 min i 0.4 mol/l perchloric acid in boiling water and enzyme hydrolysis by means of sulphatase + β-glucuronidase preparation (Glusulase®) and of β-glucuronidase preparation (Ketodase®) for 24 hrs at 37°C. Metabol te quantities in nCi/g (mean ± S.E.M 6 determinations) and average percentages from total acti ity For other particulars, see legend t T ble 22

Compound	Without hydrolysis		After acid hydrolysis		After sulphatase +β-glucuronidase hydrolysis		After β-glucuronidase hydrolysis	
	nCi/g	% of total H activity	Ci/g	% of total H activity	nCi/g	% of total H activity	nCi/g	% of total H activity
Start	0.56 + 0.14	14.9	0.10 ± 0.04	2.7	0.33 ± 0.05	8.8	0.56 ± 0.10	14 7
Conjugates	1.55 0.32	41.2	0.26 ± 0.04	7 0	0.33 ± 0.05	8.8	1.26 ± 0.22	33.2
NA	0.74 ± 0.08	19 7	1.23 ± 0.16	33.2	1 19 ± 0.15	31 7	0.87 ± 0.16	22.9
NMN	0.23 ± 0.02	6.1	1.28 ± 0.24	34.5	1.06 ± 0.19	28.3	0.22 ± 0.03	5.8
DHPG-DHMA	0.22 0.03	5.9	0.27 ± 0.03	7.3	0.25 ± 0.06	6.7	0.25 ± 0.03	6.6
MHPG-VMA	0.38 ± 0 05	10.1	0.41 ± 0.05	11 1	0.41 ± 0.05	10.9	0.38 ± 0.05	10.0
Front	0.08 ± 0.02	2.1	0.16 ± 0.03	4.3	0.18 ± 0.04	4.8	0.26 ± 0.04	0.8

$p < 0.02$ difference versus valu without hydrolysis
$p < 0.01$

Comment

In this study distinct asphyxia of the foetus prevailed This was ascertained only on the basis of the heart rate but was confirmed in one foetus by an Astrup analysis. However the heart was distinctly active 15 min after the injection in all foetuses The foetal liver was the most important tissue in NA inactivation. The greater part of the activity was discovered in the liver some 14 % of the quantity injected although the weight of the liver accounts for only 4 % of the total foetal weight, on the average. This situation probably arises on account of the route of injection. Another factor which may contribute is the large quantity of blood received by the liver when the umbilical circulation is closed

The quantity of metabolites in the liver was considerable, and the most significant mode of inactivation appeared to be conjugation with sulphuric acid Under these experimental conditions the oxidative metabolic pathway mediated by MAO is obviously impaired owing to the prevailing asphyxia. Because of this the products of such oxidative metabolism DHPG DHMA, MHPG and VMA were only found in trace amounts, even in the liver Under these conditions the metabolism seems to take place mainly *via* COMT action and *via* sulphate conjugate formation.

DISCUSSION

Paper chromatography and oxidation method

In the method employed in this study for the determination of ^{3}H NA and its metabolites after separation by paper chromatography Kaartinen's sample oxidizer (1969) was used It would also have been possible to determine the radioactivity in each fraction after chromatography by placing the appropriate stained or unstained sections directly in the scintillation liquid (see Goodall *et al.* 1964 Schanberg *et al* 1968b) This would simplify the method, but the counting efficiency would be inferior Separation by paper chromatography was combined with combustion because thereby pure, colourless samples and a good counting efficiency were obtained and because paper is a most suitable material for burning In Kaartinen's apparatus the standard deviation of the results is low 4 dpm, and the recovery is high, ca 99 % This makes the apparatus eminently suitable for the measurement of small amounts of radioactivity

The method employed is simple and easy to execute. It can thus be used for the analysis of large numbers of samples. The chromatographic separation of NA and of its metabolites achieved was well defined and the various compounds were located by staining prior to the combustion. In this method all metabolites are simultaneously carried through the same process as a result, activity is not carried into different fractions and the recoveries of all compounds were good between 94 1 and 97 1 % and showed no statistically significant variation It was shown that of any given radioactive metabolite only 0 5—1 2 could be recovered after chromatography in another metabolite fraction The reproducibility of the method is good present results reveal that the percentage of the total activity in the individual metabolite fractions was constant in seven parallel analyses of the same liver extract The coefficient of variation was greatest for the metabolit[illegible]ng the lowest activities, from 5 to 24 % according to the activ[illegible]

Running time for paper chromatography is longer than for thin layer chromatography and the method is made even more time-consuming by the long equilibration period. However thin layer chromatographic procedures are less suitable for combining with a combustion system. In the cellulose thin layer chromatographic method of Giese *et al.* (1967) which resembles the present method only marginal areas 1 cm in width are stained, which might lead to some confusion in metabolite identification. On the cellulose plates used by Giese *et al.* (1967) an aliquot of 0.2 ml only can be accommodated which restricts the possibilities of measuring low ^{3}H activities.

The paper chromatographic system described is not particularly suitable for separating DHPG from DHMA, MHPG from VMA, NA from A and NMN from MN It should also be noted that formic acid is rather rapidly esterified to butyl formate in butanol thus the chromatographic solvent is somewhat unstable and could result in variable chromatographic behaviour of samples. However the separation achieved was good and constant throughout the study and no confusion between metabolites could occur since the paper was always stained prior to combustion.

The method of Rutschmann *et al* (1965) involved a similar combination of paper chromatography and combustion. It could obviously be applied to the separation of NA and its metabolites. It is, however slower than the present method and involves a greater number of steps and would have proved cumbersome for handling large numbers of samples. It was therefore considered unsuitable for the present study

The method of Kopin *et al.* (1961) or its modifications are the most widely used for the determination of radioactive tracer labelled NA and of its metabolites (*e g* Wurtman, Kopin and Axelrod 1963 Glowinski and Iversen 1966 Landsberg and Axelrod 1968, Faiman, Meyers and Schowen 1973) In these methods, the metabolites are separated into numerous groups by a number of column chromatographic procedures and the losses of H activity in the different fractions vary considerably A method involving as many steps as this is not of particular use when the number of samples to be analysed is great, and the large variation in recovery is objectionable especially if the ^{3}H activity levels are low

Influence of asphyxia on the results

In order to maintain the umbilical circulation, fluothane was administered. Such treatment relaxes the uterus. The heart of all the foetuses was active throughout the experimental period. The influence of exteriorization of the human foetus on its circulation has been studied in this clinic (Rudolph *et al.* 1971) and the experimental arrangement used in the present study was based on their findings. They showed that the blood gas analyses of the umbilical vessels were nearly constant for 15 min that during this time, owing to asphyxia, the placental circulation is reduced to about 50 % of its original volume and this results in a redistribution of the circulation in the foetus. The proportion of the cardiac output received by the brain, adrenals and myocardium increases, while that of the lungs, intestine and kidneys decreases

In an effort to estimate the way in which the potential redistribution of the circulation may affect present results one could imagine that in the foetus remaining *in utero* (experimental arrangement II) there will be no asphyxia. In the foetoplacental unit (experimental arrangement III) a fairly strong asphyxic situation has already obviously developed during the test, and when the foetus was kept outside the uterus with its umbilical cord clamped (experimental arrangement IV) an anoxic state prevailed Judging by the ^{3}H content of the foetal tissues the results are consistent with the idea that in an asphyxic situation the quantity of blood going to the heart, at least, would increase, whereas no increase in the blood flow to the brain and adrenals has been positively observed.

However the behaviour of NA and its metabolites was basically the same in the foetoplacental unit, in the acute foetal sampling experiments carried out 1 to 5 min after injection of ^{3}H NA into the mother in the foetus *in utero* and in the absence of asphyxia. It has thus been possible to solve the problems outlined in the beginning of this report. The investigation of the ability of the foetus to conjugate ^{3}H NA and its metabolites after removal from the uterus was qualitative by its nature and anoxia was no impediment in the assessment of the results.

It is difficult to design experiments on human subjects that do not involve the added parameter of drug effects. General anaesthesia does not appear to have any effect on the rate of disappearance of ^{3}H NA from the circulation The drugs used in this study are known to pass through the placenta (Idänpään-Heikkilä, Jouppila, Puolakka and Vorne

1971 Kivalo and Saarikoski 1970, 1972 Mirkin 1973), but it is difficult to estimate what influence the use of these drugs may have had on the results.

Passage of noradrenaline and its metabolites into the foetus

The placenta may be compared functionally to a lipoprotein membrane. Fat-soluble substances pass through it more efficiently than water soluble ones, and non ionized more efficiently than ionized substances Substances with a molecular weight less than 600 pass it easily into the foetus, while for molecular weights in excess of 1000 the placenta acts as a barrier (for ref., see Ginsburg 1971 Levy and Hayton 1973 Mirkin 1973) The molecular weights of NA and its metabolites are low of the order of 200 but these compounds are hydrophilic. However on the basis of their low molecular weight it might be assumed that these compounds pass into the foetus with ease.

Passage of NA into the foetus The passage of ^{3}H NA added to the mother's circulation, into the foetus, observed in the present study was minimal and it can hardly be attributed any significance in the regulation of the foetal circulation as has been thought possible (Sandler *et al.* 1963 1964 Morgan *et al* 1972) NA metabolites, on the other hand, reach the foetus easily and rapidly which shows that the placental enzyme barrier efficiently isolates the foetus from the effect of the transmitter substances of the mother's adrenergic nervous system. This observation must be interpreted as indicating that in the placenta *in vivo* rapid deamination and O-methylation take place, as was previously indicated by *in vitro* studies (Luschinsky and Singher 1948 Iisalo and Castrén 1967 Klinge 1968 Castrén and Saarikoski 1974)

No attempt was made at estimating placental NA inactivating capacity in the present study indeed, it is obvious that the placenta has a high NA metabolizing capacity (Bekaert *et al* 1966 Thiery *et al.* 1967) Only when the quantity of CA in the circulating blood undergoes a marked increase, as in pheochromocytoma, it is conceivable that there is a risk of NA traversal in such quantities that it could have a physiological effect. Strong placental degeneration associated with pheochromocytoma (see Batts *et al.* 1974) or *e g* with toxaemia, may also possibly increase the passage of NA into the foetus especially during labour In this connection, remarkably high plasma NA concentrations were

found in some such patients (Saarikoski, Jäättelä and Ikonen unpublished data 1972) The placental and umbilical blood vessels are not sensitive to the effect of NA (Klinge, Mattila Penttilä and Jukarainen 1966 Dawes 1973) in contrast with the circulatory shunts of the foetus (Ehinger et al 1968 Boréus et al 1969 Aronson et al. 1970 Owman et al 1973) It seems to be obvious that high NA levels in the maternal blood can be harmful to the foetus mainly as a result of foetal hypoxia arising from NA induced uterine arterial vasoconstriction and uterine contractions.

NA in foetal tissues It has been shown that the uptake of ^{3}H NA into tissues after intravenous injection depends on two factors, *i.e* the proportion of cardiac output reaching the tissue and the endogenous NA content (for ref., see Axelrod 1965 Iversen 1967) In human foetal organs the NA content roughly parallels that of adult organs of other species indicating the presence of a functioning adrenergic nerve supply and of NA binding capacity at an early stage of development (Greenberg and Lind 1961 Read and Burnstock 1970 Hervonen 1971)

With the foetus *in utero* the activity passing through the placenta consisted almost totally of metabolic products of ^{3}H NA, and therefore the quantity of ^{3}H NA in the foetal tissues was indeed very low not even measurable in all instances The ^{3}H NA which passes into the foetus is taken up mainly by the liver since of its total activity after 5 min ca. 15 % consisted of conjugated ^{3}H NA. Uptake by the other foetal tissues plays a very minor role in the foetal inactivation *in utero* of ^{3}H NA administered to the mother

If a more considerable amount of ^{3}H NA enters the foetus as occurred with the foetoplacental unit experimental model and when the foetus was treated outside the uterus with its umbilical cord clamped the adrenergic uptake mechanism is obviously of significance in the inactivation of ^{3}H NA, because the greater part of the radioactivity found in the tissues under these circumstances was ^{3}H NA. Tissue uptake of ^{3}H NA, however seems to be a less important mechanism of inactivation than hepatic metabolism. This could be seen clearly in the extrauterine experimental arrangement where the quantity of radioactivity in the liver alone was on the average equal to the aggregate content of all other tissues examined. But, it is felt that with this particular arrangement the involvement of the liver is potentiated by the administration of the ^{3}H NA into the umbilical vein.

Metabolites of NA in the foetal tissues The distribution of ³H NA metabolites in the foetal tissues *in utero* was for the most part similar in all tissues studied to that in the placenta and umbilical venous blood, the predominant unconjugated metabolite was ³H MHPG-VMA. The results presented indicate that the placental metabolism is the main foetal protecting system. NA metabolites appear to have a particular affinity for the liver the concentration of ³H MHPG-VMA in the liver after 5 min was equal to that in the umbilical vein and was clearly higher than that in other foetal tissues or in the placenta, and 30 min after the administration of ³H NA to the mother the concentration of ³H MHPG-VMA in the foetal liver was distinctly higher than that in the umbilical vein, placenta and foetal tissues. These results approximate those obtained *in vitro* during rat liver perfusion. The liver accumulates metabolites of A over a prolonged period of time from the perfusing medium but not A (Lightman and Hems 1973)

With the foetoplacental system, after the addition of ³H NA to the foetal circulation 10 to 25 % of the activity was found as unconjugated metabolites in the foetal tissues. The metabolic transformation of the ³H NA was mediated mainly by the action of COMT as is the case in adult tissues also (Hertting and Axelrod 1961) This can be seen from the fact that in the tissues the concentration of ³H NMN was ca. twice that of ³H DHPG VMA, and the concentration of ³H MHPG-VMA equal or greater than that of the latter

Conjugation of NA and its metabolites in the foetus Under different experimental arrangements ³H NA, ³H NMN and ³H MHPG VMA were found in conjugated form It is obvious that ³H NA and its metabolites can be conjugated in the foetus, because the proportion of activity in the foetal liver in conjugated form was significantly higher than in the umbilical venous, umbilical arterial and maternal peripheral blood and since the amount of conjugated activity in the umbilical venous blood during the first five min after H NA administration to the mother was minimal. However the possibility cannot be completely excluded that ³H NA and its metabolites conjugated in the mother might be transported into the foetus to some extent.

The ability of the foetus to conjugate H NA and its metabolites with sulphuric acid was confirmed with the extrauterine experimental arrangement. Under these conditions with the umbilical cord clamped and the connection with the mother eliminated it was possible to measure the foetus own metabolic and NA-inactivating capacity under anoxic

SUMMARY

The material consisted of 44 foetuses (11–24 weeks) weighing between 15 and 420 g. The foetuses were obtained at the termination of pregnancy performed by a laparotomy approach.

A new method was employed for the determination of ^{3}H NA and its metabolites. The tissues were homogenized in 0.4 mol/l perchloric acid, neutralized to pH 5.4–5.8 and the metabolites separated by paper chromatography: Whatman no. 1 paper, solvent systems n-butanol: formic acid: water 70:12:15 v/v 2 days old or n-butanol: formic acid: butyl formiate: water 7:1:1:2 freshly prepared, running time 12 hours. The Rf values of NA and its principal metabolites were: NA, 0.18, NMN 0.34, DHPG 0.49, DHMA, 0.56, MHPG 0.68, VMA, 0.75 and conjugated metabolites, 0.00–0.03. The quantity of radioactivity in each metabolite fraction was determined by combustion of the paper strip after staining in Kaartinen's sample oxidizer (Kaartinen 1969) to radioactive water with subsequent liquid scintillation counting.

The recovery of radioactive NA and its metabolites (mean ± S.D.) was: NA, 94.1 ± 3.4 %, NMN 97.4 ± 2.0 %, DHPG-DHMA, 96.6 ± 3.0 %, MHPG-VMA, 94.4 ± 3.5 %.

The study consisted of four parts involving different experimental arrangements.

1. The rate of passage of NA and of its metabolites through the placenta was studied by injecting 1 ^{3}H NA of high specific activity into the mother's circulation and determining the ^{3}H NA and metabolite content of the umbilical blood 1–5 min after the injection. It was found that ^{3}H NA disappears very rapidly from the maternal circulation, the half life was estimated as ca. 30 sec. In the foetal umbilical blood radioactivity was detected 1 min after the injection and an equilibrium was established between the maternal and umbilical venous circulations within 2–3 min of the injection. The passage of ^{3}H NA into the foetus was minimal, only 10–12 % of the radioactivity in the

umbilical blood was ^{3}H NA, the rest was metabolites. ^{3}H MHPG-VMA accounted for about 50 % of the activity within 2 min after the injection and subsequently ^{3}H NA is apparently efficiently metabolized to its end product in the placenta.

2. The distribution of NA and its metabolites in the foetal tissues their storage and further metabolic processing *in utero* were studied by injecting l ^{3}H NA into the mother's circulation and taking blood and tissue samples from the mother and foetus 5 30 and 240 min after the injection

When the foetus was *in utero* the activity traversing the placenta consisted almost totally of metabolic products of ^{3}H NA and for this reason the amount of ^{3}H NA in the foetal tissues was low The ^{3}H NA which reaches the foetus was taken up primarily into the liver The ^{3}H activity in the foetus is fairly uniformly distributed between the different tissues, the concentration is lowest in the brain and highest in the aortic bodies and the liver The placental ^{3}H content was highest of all. Of the foetal tissues the liver had the highest ^{3}H content 5—30 min after the injection, but not after 240 min.

The pattern of ^{3}H NA metabolites in all the foetal tissues *in utero* was for the most part similar to that in the placenta and umbilical venous blood. ^{3}H MHPG-VMA was the predominant unconjugated metabolite in all the tissues. In the liver intestine, lungs and kidneys conjugated ^{3}H NA and ^{3}H NMN were detected 5 min after the ^{3}H NA administration to the mother and conjugated ^{3}H NMN and ^{3}H MHPG-VMA 30 min after the injection The liver retained ^{3}H NA metabolites over a prolonged period of time.

3 The uptake, storage and metabolic capacity of the foetal tissues when challenged with a large quantity of NA were studied in the foeto-placental unit by injecting dl ^{3}H NA or l ^{3}H NA, into the umbilical vein or jugular vein of the foetus. The umbilical cord was clamped 15 min after the injection and the ^{3}H NA and its metabolites in the foetal tissues were determined.

In the foetoplacental unit the quantity of radioactivity found in the brain per unit weight, was small, on the other hand the distribution of activity in the peripheral tissues was fairly uniform. In contrast with the situation in the foetus *in utero* in the foetoplacental unit the activity in the tissues consisted mainly of unmetabolized ^{3}H NA. It was present in greatest amounts in the heart, followed by the intestine, kidneys and adrenals. This result indicates that in these tissues active uptake serves as a mechanism of inactivation for circulating NA. In

the liver more than half of the activity was present in the form of conjugated metabolites. ^{3}H NA and ^{3}H NMN-sulphate. The quantity of tissue ^{3}H NMN was about twice that of ^{3}H DHPG-DHMA and ^{3}H MHPG-VMA. Thus it follows that in the foetus too O-methylation represents an important metabolic step for NA.

4 The ability of the foetus to conjugate ^{3}H NA and its metabolites with sulphuric acid was confirmed by injecting of 1 ^{3}H NA into the clomped umbilical cord. Fifteen min after injection ^{3}H NA and ^{3}H NMN-sulphate were detected in the foetal liver In this anoxic condition the disappearance of ^{3}H NA from the foetal circulation was slower than in the adult.

Under normal conditions when the foetus is *in utero* the transfer of NA from the mother to the foetus is minimal and obviously has no significance in the regulation of the foetal circulation The placenta efficiently takes care of the metabolic inactivation of any NA that may arrive from the mother both by deamination and O methylation The *foetus own NA inactivating capacity is also well developed.* The most important tissue for such inactivation is the liver which is able to metabolize NA and its metabolites through MAO and COMT activity and by catalyzing sulphate conjugation. Conjugation is also believed to take place in the lungs intestine, kidneys and adrenals.

The adrenergically innervated tissues of the human foetus have the capacity to actively take up NA already during the first half of intra uterine life, but as a mode of NA inactivation this pathway is thought to be less important than the hepatic metabolic inactivation

ACKNOWLEDGEMENTS

The present investigation was carried out at the Department of Obstetrics and Gynaecology I University of Helsinki Medical School. I am most grateful to Professor Sakari Timonen M D Head of Department I of Obstetrics and Gynaecology University of Helsinki Medical School and my superior for placing the facilities of the Department at my disposal, for his unfailing interest in my work and for his continuous encouragement

I wish to express my thanks to Professor Paavo Vara, M.D former Head of Department II of Obstetrics and Gynaecology University of Helsinki Medical School for allowing me to study patients in his clinic.

I am deeply indebted to Docent Erik Klinge, M D for many stimulating discussions, for his understanding and friendly attitude towards my work and for his valuable criticism of the manuscript. I am very grateful to Professor Matti K. Paasonen, M.D Head of the Department of Pharmacology University of Helsinki Medical School for continuous interest in my work and for his valuable support throughout the study

I wish to express my sincere thanks to Professor Olli Castrén, M.D Head of the Department of Obstetrics and Gynaecology Medical High School of Kuopio for inspiring discussions and unfailing interest in my investigations and for introducing me into obstetrical catecholamine research.

I am very much indebted to Docent Matti Härkönen, M.D for reading the manuscript and for his fundamental and constructive criticism. My friend Dr Niilo Kaartinen, M.D placed the prototype of his sample oxidizer at my disposal. I wish to thank him for this and for his help with some of my technical problems.

My gratitude is also due to my brother Architect Pentti Penttilä, for making the drawings and to Mr Matti Forss, M.Sc. for helping with problems concerning radiological protection I also owe my warm

thanks to Mrs. Marjatta Sainio M.A who helped me to collect the literature and prepare the reference list.

Mr Uljas Attila, M Sc. translated the manuscript into English and Dr Firman Martin, Ph.D revised the final text. I wish to thank them both

My wife Kirsti is entitled to my warmest gratitude for her help and patience during this investigation My best thanks are also due to our sons, Jarkko and Turkka, for their permanent optimism they never lost their belief that their father would be less occupied the next day

I wish finally to thank all my colleagues and the personnel at the operating theatre and laboratory of the Helsinki Women's Hospital for their helpful co-operation

The work was supported in part by the Finnish Cultural Foundation and by the Finnish Medical Foundation

Tapiola May 1974

Seppo Saarikoski

REFERENCES

AGATHOPOULOS, A., D. NICOLOPOULOS, N. MATHAIOPOULOS and C. PAPADATOS, Biochemical changes of catechol-O-methyltransferase during development of human liver *Pediatrics, Springfield*, 1971 *47* 125–128.

AHLQUIST R. P A study of the adrenotropic receptors. *Am. J Physiol.* 1948. *153*. 586–600.

ANTON, A. H. and D. F SAYRE, A study of the factors affecting the aluminium oxide-trihydroxyindole procedure for the analysis of catecholamines. *J Pharmac. exp. Ther* 1962. *138*. 360–375.

ARONSON, S., G. GENNSER, CH. OWMAN and N.-O SJÖBERG, Innervation and contractile response of the human ductus arteriosus. *Eur J Pharmac.* 1970. *11* 178–186.

AXELROD, J O-methylation of epinephrine and other catechols in vitro and in vivo. *Science N.Y* 1957 *126*. 400–401

AXELROD, J The metabolism storage, and release of catecholamines. *Recent Prog. Horm. Res.* 1965. *21* 597–622.

AXELROD, J W ALBERS and C. D CLEMENTE, Distribution of catechol-O-methyl transferase in the nervous system and other tissues. *J Neurochem.* 1959 *5*. 68–72.

AXELROD, J and R. TOMCHICK, Enzymatic O-methylation of epinephrine and other catechols. *J biol. Chem.* 1958. *233*. 702–705.

AXELROD, J H. WEIL-MALHERBE and R. TOMCHICK, The physiological disposition of H[3] epinephrine and its metabolit metanephrine. *J Pharmac. exp. Ther* 1959 *127* 251–256.

BAKER, J B. E., Some observations upon isolated perfused human foetal hearts. *J Physiol., Lond.* 1953. *120*. 122–128.

BARKER, J. N Fetal and neonatal cerebral blood flow *Am. J Physiol.* 1966. *210* 897–902.

BARRETT C. T., M. A. HEYMAN and A. M. RUDOLPH, Alpha and beta adrenergic receptor activity in fetal sheep. *Am. J Obstet. Gynec.* 1972. *112*. 1114–1121

BARZEL, U S., Z. BAR ILAN G RUNNEY Y LAZEBNIK, B. EDELSTEIN and A. DE VRIES, Pheochromocytoma and pregnancy simultaneous delivery by cesarean section and resection of tumor *Am. J Obstet. Gynec* 1964. *89*. 519–527

BATTS, J A. JR. N G O TOMLIN URIAN and J PASMORE, Pheochromocytoma in pregnancy case report and review of the pathophysiology *Am. J Obstet. Gynec.* 1974 *118*. 576–577

BEARD, R. W Response of human foetal heart and maternal circulation to adrenaline and noradrenaline. *Br med. J* 1962. *1* 443–446.

BEKAERT S., F BARBIER, W VAN GEERTRUYDEN and M. T DERY Une association rare phéochromocytome et grossesse. *Acta clin. belg* 1966. *21* 125–135.

BERGSTRÖM, S. and G. HANSSON The use of Amberlite IRC-50 for the purification of adrenaline and histamine. *Acta physiol. scand.* 1951 *22*. 87–92.

BERNARD, C. and Y. M. GARGOUIL, Etude électrophysiologique de la sensibilité aux catécholamines du cœur de Rat lors de la croissance embryonnaire. *C. r. Séanc. Soc. Biol.* 1967 *161* 2600–2605.

BERTLER, Å., Occurrence and localization of catechol amines in the human brain. *Acta physiol scand.* 1961 *51* 97–107

BERTLER, Å., A. CARLSSON and E. ROSENGREN A method for the fluorimetric determination of adrenaline and noradrenaline in tissues. *Acta physiol. scand.* 1958. *44*. 273–292.

BLAIR, R. G Phaeochromocytoma and pregnancy: report of a case and review of 51 cases. *J Obstet. Gynaec Br Commonw.* 1963. *70* 110–119.
BLASCHKO, H., D RICHTER and H. SCHLOSSMANN, The inactivation of adrenaline. *J Physiol.*, Lond. 1937 *90*, 1–17 ( )
BLASCHKO, H., D. RICHTER and H. SCHLOSSMANN The oxidation of adrenaline and other amines. *Biochem. J* 1937 *31* 2187–2196. (b)
BOJANEK, J K. BOZKOWA and St. KUREEPA, The HTP decarboxylase and MAO activities and 5HT level in subcellular fractions of the liver during development. *Biologia Neonat.* 1965/66. 9. 203–214
BORÉUS, L. O. T MALMFORS, D M. McMURPHY and L. OLSON, Demonstration of adrenergic receptor function and innervation in the ductus arteriosus of the human fetus. *Acta physiol. scand.* 1969 *77* 316–321
BORN G. V R. G. S. DAWES and J C. MOTT Oxygen lack and autonomic nervous control of the foetal circulation in the lamb. *J Physiol.*, Lond. 1956. *134* 149–166.
BRUNDIN, T Catecholamines in the preaortal paraganglia of fetal rabbits. *Acta physiol. scand.* 1965. *64* 287–288.
BRUNDIN, T., Studies on the preaortal paraganglia of newborn rabbits. *Acta physiol. scand.* 1966. *70* Suppl. 290. Diss. Stockholm 1966.
BRUNJES, S., E. CASTNER and J HODOMAN, Catecholamine metabolism in newborn infants. *Clin. Res* 1964 *12*. 88.
CAMPBELL, A. G M., G. S DAWES, A. P FISHMAN and A. I. HYMAN, Regional redistribution of blood flow in the mature fetal lamb. *Circulation Res* 1967 *21* 229–235.
CASTRÉN, O Urinary excretion of noradrenaline and adrenaline in late normal and toxemic pregnancy effect of rest, work, and reserpine treatment. *Acta pharmac. tox.* 1963, Suppl. 2. Diss. Turku 1963.
CASTRÉN O N KAARTINEN and S. SAARIKOSKI, The metabolism of H³-noradrenaline in human placental and foetal tissue homogenates. *Annls Chir Gynaec Fenn.* 1968. *57* 405–409.
CASTRÉN, O and S. SAARIKOSKI, The effect of some different factors on the metabolism of H³-noradrenaline in placental, adnexal and foetal tissue homogenates. *Acta obstet. gynec. scand.* 1969 *48*. Suppl. 3. 151–157
CASTRÉN, O and S. SAARIKOSKI, The simultaneous function of catechol-O-methyltransferase and monoamine oxidase in human placenta. *Acta obstet. gynec. scand.* 1974 *53*. 41–47
COHEN, G., B. HOLLAND, J SHA and M. GOLDENBERG Plasma concentrations of epinephrine and norepinephrine during intravenous infusions in man. *J clin. Invest.* 1959 *38*. 1935–1941
COMLINE, R. S. and M. SILVER, The release of adrenaline and noradrenaline from the adrenal glands of the foetal sheep. *J Physiol.* Lond. 1961 *156*. 424–444
COMLINE, R. S and M. SILVER, Development of activity in the adrenal medulla of the foetus and new-born animal. *Br med. Bull* 1966. *22*. 16–20.
COMLINE, R. S. I. A. SILVER and M. SILVER, Factors responsible for the stimulation of the adrenal medulla during asphyxia in the foetal lamb. *J Physiol.* Lond. 1965. *178*. 211–238.
CONDORELLI, S. and E. V CONCI, Passagio placentare di alcune amine adrenergiche marcate: studio sugli ovini. *Acta anaesth.*, Padova 19 0. *21* 539–547
CONCI, E. V and S. CONDORELLI, Placental transfer of catecholamines the pregnant sheep. In. *Bases fondamentales de l'anesthésie et de la réanimation obstétricales* ed. G.-G Nahas, J.-C. Salamagne, P Viars & G Vourc'h. Publ. Société Française d'Anesthésie, d Analgésie et de Réanimation. P 329–342. Paris, Librairie Arnette, 1973. (Journées d enseignement post-universitaire 1972.)
COUPLAND, R. E. The prenatal development of the abdominal para-aortic bodies in man. *J Anat* 1952. *86*. 357–372
COUPLAND, R. E. On the morphology and adrenaline-noradrenaline content of chromaffin tissue *J Endocr* 1953. 9. 194 203.
CRAWFORD, T B. B., Derivatives of adrenaline and noradrenaline in an extract of an adrenal medullary tumour *Biochem. J* 1951 *48*. 203–208
DAVISON A N Physiological role of monoamine oxidase *Physiol Rev.* 1958. *38*. 729–747
DAWES, G S. *Foetal and neonatal physiology comparative study of the changes at birth.* Chicago, Year Book Publishers, 1968.
DAWES, G S. Drug equilibration within the foetal circulation. In. *Bases fondamentales de l'anesthésie et de la réanimation obstétricales* ed. C.-G. Nahas, J.-C. Salamagne, P Viars & C Vourc'h.

Publ. Société Française d Anesthésie, d' Analgésie et de Réanimation. P 255–259 Paris,
Librairie Arnette, 1973. (Journées d'enseignement post-universitaire 1972.)
Dawes, G. S., H. E. Fox, B. M. Leduc, G. C. Liggins and R. T Richards, Respiratory
movements and rapid eye movement sleep in the foetal lamb. *J Physiol.*, Lond. 1972. *220*
119–143.
Dornhorst A. C. and I. M. Young, The action of adrenaline and noradrenaline on the
placental and foetal circulations in the rabbit and guinea-pig. *J Physiol.*, Lond. 1952
118. 282–288.
Dutton, G J Glucuronide synthesis in foetal liver and other tissues. *Biochem. J* 1959 *71*
141–148.
Ehinger, B., G. G [illegible], Ch. Owman, H. Persson and N.-O Sjöberg, Histochemical
and pharmacological studies on amine mechanisms in the umbilical cord, umbilical vein
and ductus venosus of the human fetus. *Acta physiol. scand.* 1968. *72*. 15–24
Elmadjian F E. T Lamson and R. Neri, The nature of adrenaline and noradrenaline
in normal human urine. *J clin. Endocr Metab.* 1956. *16*. 216–221
Esmbanino, G. and B. Westin, Experimental studies of the human fetus in prolonged as-
phyxia. *Acta physiol. scand.* 1954 *31* 359–375.
Euler, U S. von and U Hamberg l-noradrenaline in the suprarenal medulla. *Nature*
Lond. 1949. *163*. 642–643.
Euler, U S. von, S. Hellner Björkman and I. Orwén, Diurnal variations in the excre-
tion of free and conjugated noradrenaline and adrenaline in urine from healthy subjects.
Acta physiol. scand. 1955. *33*. Suppl. 118. 10–16.
Fadian, M. D., M. B. Myles and R. L. Schoven Post-mortem degradation kinetics of brain
norepinephrine. *Biochem. Pharmac.* 1973. *22*. 2171–2180.
Friedman W F P E. Pool, D Jacobowitz, S. C. Seagren and E. Braunwald, Sym
pathetic innervation of the developing rabbit heart. *Circulation Res.* 1968. *23*. 25–32.
Gaffney T E., R. L. Burkey and S. Woronkow Catecholamine content of the pregnant
and nonpregnant human uterus. *Obstet. Gynec.*, N Y 1965. *25*. 340–342.
Gennser, G. and E. Nilsson, Response to adrenaline, acetylcholine and change of contrac
tion frequency in early human foetal hearts. *Experientia* 1970. *26*. 1105–1107
Gennser, G. and W v Studnitz, Monoamine oxidase- and catechol-O-methyltransferase
activity in umbilical vessels of the human fetus. *Experientia* 1969 *25*. 980–981 ( )
Gennser, G. and W v Studnitz, Monoamine oxidase, catechol-O-methyltransferase and
phenylethanolamine-N-methyltransferase activity in para-aortic tissue of the human fetus.
Scand. J clin. Lab. Invest. 1969. *24* 169–171 (b)
Gehr, J E. Ritter and N. Matussek, Quantitative estimation of H³-norepinephrine and
its metabolites by thin-layer chromatography *Life Sci.* 1967 *6*. 1975–1982.
Ginsburg, J Placental drug transfer A. *Rev. Pharmac* 1971 *11* 387–408.
Girard, H. (présentée par E. Wolff) Action de l adrénaline sur la circulation foetale du Rat
et du Cobaye. *C. r. hebd. Séanc. Acad. Sci.*, Paris 1965. *261* 1099–1101
Glowinski, J J Axelrod, I. J Kopin and R. J Wurtman, Physiological disposition of
H-norepinephrine in the developing rat. *J Pharmac exp Ther* 1964 *146*. 48–53.
Glowinski, J and L. Iversen, Regional studies of catecholamine in the rat brain — I; The
disposition of [³H]norepinephrine, [³H]dopamine and [³H]dopa in various regions the
brain. *J Neurochem.* 1966. *13*. 655–669
Goldstein, M., A. J Friedhoff and C. Simmons, A method for the separation and estimation
of catechol amines in urine. *Experientia* 1959. *15*. 80–81
Goodall, McC., H. Alton and L. Rosen Normetadrenaline metabolism in man. *Biochem
Pharmac.* 1964 *13*. 703–711
Goodall, McC. and A. W Diddle, Epinephrine and norepinephrine in pregnancy a com-
parative study of the adrenal gland and catechol output in different species of animal
and man. *Am. J Obstet. Gynec.* 1971 *111* 896–904
Goodall, McC., N Kirshner and L. Rosen, Metabolism of noradrenaline in the human.
J clin. Invest. 1959. *38*. 707–714
Greenberg, R. E. and J Lind, Catecholamines in tissues of the human fetus. *Pediatrics*
Springfield 1961 *27* 904–911
Häggendal, J., On the use of strong exchange resins for determinations of small amounts
of catechol amines. *Scand. J clin. Lab. Invest.* 1962. *14* 537–544
Häggendal, J An improved method for fluorimetric determination of small amounts of
adrenaline and noradrenaline in plasma and tissues. *Acta physiol. scand.* 1963 *59* 242–254 ( )

HAGGENDAL, J., The presence of conjugated adrenaline and noradrenaline in human blood plasma. *Acta physiol. scand.* 1963. *59* 255–260 (b)
HAMELBERG, W., J. H. SPROUSE, J. E. MAHAFFEY and J. A. RICHARDSON, Catechol amine levels during light and deep anesthesia. *Anesthesiology* 1960. *21* 297–302.
HARE, M. L. C., Tyramine oxidase. I. A new enzyme system in liver. *Biochem. J.* 1928. *22* 968–979
HART, S. L. and M. S. MIR, Adrenoreceptors in the human foetal small intestine. *Br. J. Pharmac. Chemother.* 1971 *41* 567–569 ( )
HART, S. L. and M. S. MIR, Adrenoceptors in the human foetal colon. *Br. J. Pharmac. Chemother.* 1971 *42* 662P–663P (b)
HARTIALA, K. J. V. and M. PULKKINEN, Studies on detoxication mechanisms; glucuronide synthesis in foetal rabbit. *Annls Med. exp. Biol. Fenn.* 1955. *33* 246–248.
HERTTING, G. and J. AXELROD, Fate of tritiated noradrenaline at the sympathetic nerve-endings. *Nature* Lond. 1961 *192* 172–173.
HERTTING, G., J. AXELROD and G. WHITBY, Effect of drugs on the uptake and metabolism of H³-norepinephrine. *J. Pharmac. exp. Ther.* 1961 *134* 146–153.
HERVONEN, A., Development of catecholamine-storing cells in human fetal paraganglia and adrenal medulla, a histochemical and electron microscopical study. *Acta physiol. scand.* 1971 Suppl. 368. Diss. Helsinki 1971
HERVONEN, A. and O. KORKALA, The effect of hypoxia on the catecholamine content of human fetal abdominal paraganglia and adrenal medulla. *Acta obstet. gynec. scand.* 1972. *51* 17–24
HEYMANN, M. A. and A. M. RUDOLPH, Effect of exteriorization of the sheep fetus on its cardiovascular function. *Circulation Res.* 1967 *21* 741–745.
HOLTZ, B., Noradrenaline and adrenaline in mammalian tissues; distribution under normal and pathological conditions with special reference to the endocrine system. *Acta physiol. scand.* 1951 *25* Suppl. 92.
HYYPPÄ, M., Hypothalamic monoamines in human fetuses. *Neuroendocrinology* 1972. *9* 257–266.
IDÄNPÄÄN-HEIKKILÄ, J. E., P. I. JOUPPILA, J. O. PUOLAKKA and M. S. VORNE, Placental transfer and fetal metabolism of diazepam in early human pregnancy. *Am. J. Obstet. Gynec.* 1971 *109* 1011–1016.
IGNARRO, L. J. and F. E. SHIDEMAN, Appearance and concentrations of catecholamines and their biosynthesis in the embryonic and developing chick. *J. Pharmac. exp. Ther.* 1968. *159* 38–48. ( )
IGNARRO, L. J. and F. E. SHIDEMAN, Catechol-O-methyl transferase and monoamine oxidase activities in the heart and liver of the embryonic and developing chick. *J. Pharmac. exp. Ther.* 1968. *159* 29–37 (b)
IGNARRO, L. J. and F. E. SHIDEMAN, Norepinephrine and epinephrine in the embryo and embryonic heart of the chick. uptake and subcellular distribution. *J. Pharmac. exp. Ther.* 1968. *159* 49–58. (c)
IHALO, E. and O. CASTRÉN, The enzymatic inactivation of noradrenaline in human placental tissue. *Annls Med. exp. Biol. Fenn.* 1967 *45* 253–257
IVERSEN, L. L., The uptake of noradrenaline by the isolated perfused rat heart. *Br. J. Pharmac. Chemother.* 1963. *21* 523–537
IVERSEN, L. L. *The uptake and storage of noradrenaline in sympathetic nerves.* Cambridge University Press, 1967
IVERSEN, L. L., J. DE CHAMPLAIN, J. GLOWINSKI and J. AXELROD, Uptake, storage and metabolism of norepinephrine in tissues of the developing rat. *J. Pharmac. exp. Ther.* 1967 *157* 509–516.
JAMES, W. O. Demonstration and separation of noradrenaline, adrenaline and methyladrenaline. *Nature* Lond. 1948. *161* 851–852.
JAMES, W. O. and N. KILBEY Separation of noradrenalin and adrenalin. *Nature* Lond. 1950. *166* 67–68.
JOELSSON, I. and M. D. BARTO The effect of blockade of the beta receptors of the sympathetic nervous system of the fetus, preliminary report. *Acta obstet. gynec. scand.* 1969. *48* Suppl. 3 75–79
JONES, H. E. *The physics of radiology* 2. ed. Springfield, Thomas, 1961
JONGE, H. DE, pp 486–487 in: *Inleiding tot de medische statistiek*, deel II 2. ed. Verhandeling an het Nederlands Instituut voor Praeventieve Geneeskunde, Leiden, 1964

KAARTINEN, N., A new oxidation method for the preparation of liquid scintillation samples. *Packard tech. Bull.* 1969 Number 18. (Packard Instrument Company 2200 Warrenville Rd. Downers Grove Ill. 60515.)

KARKI, N., R. KUNTZMAN and B. B. BRODIE, Storage, synthesis, and metabolism of monoamines in the developing brain. *J. Neurochem.* 1962. 9. 53–58.

KERCHTARIS, N., McG GOODALL and L. ROSEN. The effect of iproniazid on the metabolism of dl-epinephrine-2-C¹⁴ in the human. *J. Pharmac. exp. Ther.* 1959, 127 1–7

KIVALO, I. and S. SAARIKOSKI, Quantitative measurements of placental transfer and distribution of radioactive atropine in fetus. *Annls Chir. Gynaec. Fenn.* 1970. 59 80–84

KIVALO, I. and S. SAARIKOSKI. Placental transmission and foetal uptake of ¹⁴C-dimethyltubocurarine. *Br. J. Anaesth.* 197.. 44 557–561

KLINGE, E., O-methylation of adrenaline in the human placenta. *Scand. J. clin. Lab. Invest.* 1968. 21 Suppl. 101 83–84

KLINGE, E., M. J. MATTILA, O. PENTTILA and E. JUKARAINEN. Influence of drugs on vasoactive peptides and amines in perfused human placenta. *Annls Med. exp. Biol. Fenn.* 1966. 44 369–3 3.

KLINGE, E., O. PENTTILA and A. TEIRAKI, The content of 5-hydroxytryptamine in human uterine and placental tissue and their 5-hydroxytryptophan decarboxylase and monoamine oxidase activities in normal and toxaemic pregnancies. *Acta obstet. gynec. scand.* 1961 41. 107–114

KOPIN, I. J., J. AXELROD and E. GORDON, The metabolic fate of H³-epinephrine and C¹⁴-metanephrine in the rat. *J. biol. Chem.* 1961 236. 2109–2113.

LABROSSE, E. H., J. AXELROD, I. J. KOPIN and S. S. KETY. Metabolism of 7-H³-epinephrine-d-bitartrate in normal young men. *J. clin. Invest.* 1961 40. 253–260.

LAHI, E., A. PEKKARINEN, S. SAARIKOSKI and I. SURAMO, The effects of pregnancy on the content of noradrenaline and adrenaline in the heart, spleen and kidney of guinea pigs and rats and on the histochemical fluorescence reaction of adrenergic nerve fibres. *Acta pharmac. tox.* 1967 25. Suppl. 4 12–13.

LANDSBERG, L. and J. AXELROD, Reduced accumulation of H³-epinephrine in the rat heart following hypophysectomy. *Endocrinology* 1968. 82 175–178.

LANGEMANN H., Weitere Untersuchungen über das Vorkommen der Cholinesterase und der Monoaminoxydase in menschlichen Organen, 2. Mitteilung über Bestimmungen der Fermentaktivität in normalen und pathologisch-veränderten Organen. *Helv. physiol. pharmac. Acta.* 1944. 2. 367–375.

LEMPINEN M., Extra-adrenal chromaffin tissue of the rat and the effect of cortical hormones on it. *Acta physiol. scand.* 1964. 62 Suppl. 231 Diss. Helsinki 1964.

LEVINE, R. J. and A. SJOERDSMA, Monoamine oxidase activity in human tissues and intestinal biopsy specimens. *Proc. Soc. exp. Biol. Med.* 1962. 109. 225–227

LEVY G and W. L. HAYTON. Pharmacokinetic aspects of placental drug transfer. *In: Fetal Pharmacology*, ed. L. Boréus. P 29–39. New York, Raven Press, 1973

LIGHTMAN S. L. and D. A. HEMS, Metabolism of adrenaline in the isolated perfused liver of the rat. *Biochem. Pharmac.* 1973. 22. 2419–2423

LIPP J. A. M. and A. M. RUDOLPH, Sympathetic nerve development in the rat and guinea-pig heart, *Biologia Neonat.* 1972. 21 76–82.

LUND, A., Elimination of adrenaline and noradrenaline from the organism. *Acta pharm. tox.* 1951 7 297–308

LUSCHINSKY H. L. and H. O. SINGHER, Identification and assay of monoamine oxidase in the human placenta. *Archs Biochem.* 1948. 19 95–97

McMURPHY D. M. and L. O. BORÉUS, Pharmacology of the human fetus: adrenergic receptor function in the small intestine. *Biologia Neonat.* 1968. 13. 325–339.

MARGOLIS, F. L., J. ROTH and A. JOST. Norepinephrine methylation in fetal rat adrenals. *Science N.Y.* 1966. 154 275–276.

MARIA, F. J. DE, Placental monoamine oxidase in normal and toxemic patients. *Am. J. Obstet. Gynec.* 1964 88. 490–494

MATSUOKA, D. T., W. DRELL, H. F. SCHOTT, A. F. ALGARAZ and E. C. JAMES, Separation of catecholamines and their metabolites. *Analyt. Biochem.* 1963. 5. 426–432.

MIRKIN B. L., Drug distribution in pregnancy. In: *Fetal Pharmacology*, ed. L. Boréus. P 1–27 New York, Raven Press, 1973.

MORGAN, C. D., M. SANDLER and M. PANIGEL, Placental transfer of catecholamines in vitro and in vivo. *Am. J. Obstet. Gynec.* 1972. 112. 1068–1075.

NICOLOPOULOS, D., A. AGATHOPOULOS, C. DANELATHOU-ATHANASIADOU and M. BAFATAKI, Urinary excretion of phenolic and indolic compounds, metacatecholamines, and VMA by full-term and premature infants. *Pediatrics* Springfield 1968. *41* 777—783.
NIEMINEVA, K. and A. PEKKARINEN, The noradrenaline and adrenaline content of human fetal adrenal glands and aortic bodies. *Annls Med. exp. Biol. Fenn.* 1952. *30* 274—286.
NIKKI, P S. TAKKI, T TAMMISTO and A. JÄÄTTELÄ, Effect of operativ stress on plasma catecholamine levels. *Int. clin. Res.* 1972. *4* 146—151
NOBIN, A. and A. BJÖRKLUND, Topography of the monoamine neuron systems in the human brain as revealed in fetuses. *Acta physiol. scand.* 1973. Suppl. 388.
NOWICK, W J Effect of oxygen tension on monoamine oxidase activity *Biochem. Pharmac.* 1966. *15.* 1009—1012.
OWMAN C., S. ARONSON G. GENNSER and N-O SJOBERG Histochemical and pharmacological evidence of amine mechanisms in human fetal vascular shunts. In: *Fetal Pharmacology* ed. L. Boréus. P 179—191 New York, Raven Press, 1973.
OWMAN, CH., E. ROSENGREN and N-O SJÖBERG, Adrenergic innervation of the human female reproductive organs: histochemical and chemical investigation. *Obstet. Gynec* N.Y 1967 *30*. 763—773.
PARDI, G M. TIENGO, A. UDERZO, L. SALMOIRAGHI and G B. CANDIANI, Azione dell'adrenalina della noradrenalina sul sistema cardiovascolare del fet di pecora. *Annali Ostet. Ginec.* 1969 *91* 231—244
PARTRIDGE, S. M., with a note by R. G. WESTALL, Filter-paper partition chromatography of sugars; 1 General description and application to the qualitative analysis of sugars I apple juice egg white and foetal blood in sheep. (WESTALL, R. G., Addendum. Note on the behaviour of inorganic salts on the filter paper partition chromatogram.) 2. An examination of the blood group specific substance from hog gastric mucin and the specific polysaccharide of *Bacterium Dysenteriae* (Shiga) *Biochem J* 1948. *42*, 238—253.
PEKKARINEN, A. Studies on the chemical determination, occurrence and metabolism of adrenaline in the animal organism. *Acta physiol. scand.* 1948. *16.* Suppl. 54 Diss. Helsinki 1948.
PEKKARINEN, A. and O CASTRÉN, Excretion of vanilmandelic acid (VMA) in the third trimester of normal and toxaemic pregnancy and other clinical conditions. *Annls Chir Gynaec. Fenn.* 1968. *57* 373—381
PEAN, R. G., The effect of age species and adrenaline on the recovery of isolated tria from anoxia. *Br J Pharmac. Chemother* 1970. *39* 309—316.
PLACE, V S. and F CAMO E, Mono- di- amino-ossidasi nella placenta nell utero di donna nel sangue di feto umano. *Boll Soc. ital. Biol. sper* 1947 *23*. 933—934
POTTER, W P DE, R F VOCHTEN and A. F DE SCHAEPDRYVER, Thi layer chromatography of catecholamines and their metabolites. *Experientia*, 1965. *25*. 482—483.
PULKKINEN, M. O Hormone- sulphatase relationsh ps and sulphate conjugation i the foetus and placenta. *Acta physiol scand.* 1963. *59.* Suppl. 213. 124
PULKKINEN M. O Sulphate conjugation during development, I human. at and guinea pig. *Acta physiol. scand.* 1966. *66*. 115—119.
PUR LE, M. J and I M. JAMES, Observations on the control of cerebral blood flow in the sheep fetus and newborn lamb. *Circulation Res* 1969. *25.* 651—667
READ, J B. and G BURNSTOCK, Development of the adrenergic innervation and chromaffin cells the human fetal gut. *Devl Biol.* 1970. *2*. 513—534
RENO P W NEWMA and C. WOOD, Autonomic control of fetal heart rate *Int. J Obstet. Gynec* 1969. *105*. 949—953
RENTZHOG, L. Double isotope dilution deri ativ technique for measuremen of catecholamines. *Acta physiol. scand.* 1972. Suppl. 377 Diss. Uppsala 1972
RICHTER, D The inactivation of adrenaline in i o in man. *J Physiol.*, Lond. 1940. *98* 361 374
RICHTERICH R. *Klinische Chemie Theorie und Praxis*, pp. 17—36. 2. ed. Basel Karger 1968.
ROBINSON, A Histochemistry of monoamine oxidase the developing rat brain. *J Neurochem.* 1967 *14* 1083 1089
ROS I. J (présentée par R. COURRIER Influence de la corticosurrénale chez le fœtus de Rat C *hebd. Séanc Acad Sci.* Paris 1965. *260*. 1267—1270.
R DOLPH A M The course and distribution of the foetal circulation. In. *Foetal autonomy Ciba Foundation symposium*, ed. G E W Wolstenholme & M. O'Connor P 147—161 London, Churchill 1969

RUDOLPH, A. M. and M. A. HEYMANN, The circulation of the fetus in utero; methods for studying distribution of blood flow, cardiac output and organ blood flow. *Circulation Res.* 1967 *21* 163–184. (a)
RUDOLPH, A. M. and M. A. HEYMANN, Validation of the antipyrine method for measuring fetal umbilical blood flow. *Circulation Res.* 1967 *21* 185–190. (b)
RUDOLPH, A. M. and M. A. HEYMANN, Control of the foetal circulation. In: *Foetal and neonatal physiology; proceedings of the Sir Joseph Barcroft Centenary Symposium, held at the Physiological Laboratory Cambridge 25 to 27 July* 1972. P 89–111. Cambridge, University Press, 1973.
RUDOLPH, A. M., M. A. HEYMANN, K. A. W. TERAMO, C. T. BARRETT and N. C. R. RÄIHÄ, Studies on the circulation of the previable human fetus. *Pediat. Res.* 1971 *5* 452–465.
RUTHERMAN, J. W., PACHA, F. KALBERER and E. SCHREIER, A method for the study of drug influenced catecholamine metabolism using L-DOPA-(2, 5, 6-H^3). In: *Isotopes in experimental pharmacology*, ed. L. J. Roth. P 295–306. Chicago, University Press, 1965.
SAARIKOSKI, S., *Unpublished observations* 1972.
SAARIKOSKI, S., Placental transfer, distribution and metabolism of ^{3}H-norepinephrine in human fetus in vivo. In: *Nordisk Forening for Obstetrik og Gynekologi, official transactions* 11: *sixteenth congress Aarhus 29–30 June 1 July 1972*, ed. M. Ingerslev & O. Rosen Steenstrup. P 38–41. Aarhus, Universitetsforlaget, 1972. (The Scandinavian Association of Obstetricians and Gynecologists.)
SAARIKOSKI, S. and O. CASTRÉN, Distribution and metabolism of H^3-noradrenaline in various tissues of the human foetus in the foetoplacental unit. *Acta obstet. gynec. scand.* 1971 *50* Suppl. 9. 60.
SAARIKOSKI, S., M. IKONEN and A. JÄÄTTELÄ, *Unpublished data* 1972.
SADONE, L. and U. ARBOTTA, Rapporti tra serotonina e monoaminossidasi placentari nella gestosi eclamptica. *Annali Ostet. Ginec.* 1966. *88*. 81–84.
SANDLER, M. and J. COVENEY, Placental monoamine-oxidase activity in toxaemia of pregnancy. *Lancet* 1962. *I* 1096–1098.
SANDLER, M., C. R. J. RUTHVEN, S. F. CONTRACTOR, C. WOOD, R. T. BOOTH and J. H. M. PINKERTON, Transmission of noradrenaline across the human placenta. *Nature* Lond. 1963. *197* 598.
SANDLER, M., C. R. J. RUTHVEN and C. WOOD, Metabolism of C^{14}-norepinephrine and C^{14}-epinephrine and their transmission across the human placenta. *Int. J. Neuropharmac.* 1964. *3*. 123–128.
SCHANBERG, S. M., G. R. BREESE, J. J. SCHILDKRAUT, E. K. GORDON and I. J. KOPIN, 3-methoxy-4-hydroxyphenylglycol sulfate in brain and cerebrospinal fluid. *Biochem. Pharmac.* 1968. *17* 2006–2008. (a)
SCHANBERG, S. M., J. J. SCHILDKRAUT, G. R. BREESE and I. J. KOPIN, Metabolism of normetanephrine-H in rat brain — identification of conjugated 3-methoxy-4-hydrophenylglycol as the major metabolite. *Biochem. Pharmac.* 1968. *17* 247–254. (b)
SEGURA-CARDONA, R. and K. SOEHRING, Dünnschichtchromatographischer Nachweis kleiner Mengen von Katecholaminen und deren Derivaten. *Medna exp.* 1964. *10*. 251–257.
SHARMAN, D. F., The catabolism of catecholamines. *Br. med. Bull.* 1973. *29* 110–115.
SHAW, F. H., The estimation of adrenaline. *Biochem. J.* 1938. *32*. 19–25.
SHEPHERD, D. M. and G. B. WEST, Noradrenaline and the suprarenal medulla. *Br. J. Pharmac. Chemother.* 1951. *6* 665–674.
SHEPHERD, D. M. and G. B. WEST, Noradrenaline and accessory chromaffin tissue. *Nature*, Lond. 1952. *170* 42–43.
SJÖBERG, N.-O., The adrenergic transmitter of the female reproductive tract: distribution and functional changes. *Acta physiol. scand.* 1967. Suppl. 305. Diss. Lund 1967.
СКОРОБОГАТА, Т. Г., Ю. О. ДРЕЛЬ, Активність моноамінооксидази в печінці плоді людини в процесі антенатального розвитку. *Український біохімічний журнал* 1973. 45. №4. 457–461.
SKOROBOGATA, T. H. and Ju. O. DREL, Monoamine oxidase activity in liver human fetuses in the process of antenatal development. *Ukr. biokhim. Zh.* 1973. 45. No 4. 457–461.
STUDNITZ, W. von, Catecholamine and adenosine triphosphate content of human foetal adrenals. *Scand. J. clin. Lab. Invest.* 1968. *22*. 185–188.
TAMMISTO, T., A. JÄÄTTELÄ, P. NIKKI and S. TAKKI, Effect of pentazocine and pethidine on plasma catecholamine levels. *Ann. clin. Res.* 1971. *3*. 22–29.
THERY, M., R. M. J. DEBOIS, H. E. van KETS, A. F. DE SCHAEPDRYVER, P. J. BERNARD,

S. A. L. Bekaert C. M. J Hooft F Darom, G Roll and H. J L. Rolls Pheochromocytoma i pregnancy including observations on the physiopathology and the enzymic histochemistry of this tumor *Am. J Obstet. Gynec.* 1967 *97* 21—29

Thompson R. H. S. and A. Tickner, Observations on the mono-amine oxidase activity of placenta and uterus. *Biochem. J* 1949. *45*. 125—130.

Tmaari, A., 5-hydroxytryptamine, 5-hydroxytryptophan decarboxylase and monoamine oxidase during foetal and postnatal development in the guinea-pig. *Acta physiol. scand.* 1966. *67* Suppl. 265. Diss. Helsinki 1966

Towell, M. E. Catecholamine depletion and the response to asphyxia in the fetal guinea pig. *Biol Neonate* 1971 *18*. 212—224

Towell, M. E., A. I Hyman, L. S. James, O. S. Steinsland, E. C. Gerst and K. Adamsons Jr., Reserpine administration during pregnancy; effects upon adrenal catecholamine stores and thermoregulation in the newborn. *Am. J Obstet. Gynec.* 1965. *92*. 711—716.

Vapaavuori, E.K., E. A. A. Shinebourne, R. L. Williams, M. A. Heymann and A. M Rudolph, Development of cardiovascular responses to autonomic blockade in intact fetal and neonatal lambs. *Biol. Neonate* 1973. *22*. 177—188.

Vendsalu A., Studies on adrenaline and noradrenaline i human plasma. *Acta physiol. scand.* 1960. *49*. Suppl. 173 Diss. Lund 1960.

Weil Malherbe, H. and A. D Bone, The chemical estimation of adrenaline-like substances in blood. *Biochem. J* 1952. *51* 311—318.

Wengle, B., Studies on ester sulphates; 21 On sulphate conjugation in foetal human tissue extracts. *Acta Soc Med. upsal* 1964 *69*. 105—124

Wurtman, R. J and J Axelrod, Control of enzymatic synthesis of adrenaline in the adrenal medulla by adrenal cortical steroids. *J biol. Chem.* 1966. *241* 2301—2305.

Wurtman, R. J., I J Kopin and J Axelrod, Thyroid function and the cardiac disposition of catecholamines. *Endocrinology* 1963. *73*. 63—74.

Zuckerkandl, E., Ueber Nebenorgane des Sympathicus im Retroperitonaealraum des Menschen. Verh. anat. Ges., Jena *Ergänzungsh. z. Band XIV des Anat. Anz.* 1901 95—107

Zuspan, F P., Urinary excretion of epinephrine and norepinephrine during pregnancy *J clin. Endocr Metab* 1970 *30*. 357—360.

Zuspan F P., G. H Nelson, R. P Ahlquist and S. Williams, Alterations of urinary epinephrine and norepinephrine the antepartum intrapartum and postpartum periods. *Am. J Obstet Gynec.* 1967 *99* 709—721

Zuspan F P W H Whaley G H Nelson and R. P Ahlquist Placental transfer of epinephrine; I Maternal-fetal metabolic alterations of glucose and nonesterified fatty acids. *Am. J Obstet. Gynec* 1966. *95*. 284—289.

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 422

CARDIORESPIRATORY RESPONSE TO UNILATERAL PULMONARY ARTERY OCCLUSION

AN EXPERIMENTAL STUDY IN THE DOMESTIC FOWL

By

ESKO LÄNSIMIES

TURKU 1974

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 422

From the Cardiorespiratory Research Unit (Head: Professor Tuomas Peltonen) and the Department of Physiology (Head: Professor Kaarlo Hartiala) University of Turku Finland

CARDIORESPIRATORY RESPONSE TO UNILATERAL PULMONARY ARTERY OCCLUSION

AN EXPERIMENTAL STUDY IN THE DOMESTIC FOWL

By

ESKO LÄNSIMIES

CONTENTS

		Page
1	Abbreviations and symbols	4
2	Introduction	5
3	Review of the literature	8
	3 1 Cardiorespiratory parameters of the domestic fowl	8
	3 2 Cardiorespiratory responses to stress in the domestic fowl	9
4	Purpose of the present study	15
5	Material and methods	16
	5 1 Operative procedures	16
	5 2 Cardiorespiratory recordings	17
	5 3 Statistical methods	18
	5 4 Morphological methods	19
6	Results	
	6 1 Cardiorespiratory response to occlusion of the left pulmonary artery	21
	6 2 Morphological response to the occlusion	24
7	Discussion	27
8	Abstract	33
9	Acknowledgements	34
10	References	37
11	Appendix	I
	Tables	II - VIII
	Figures	IX - XXV

ABBREVIATIONS AND SYMBOLS

a = arterial

b w = body weight

CO_2 = carbon dioxide

cm = centimeter

d = day days

ECG = electrocardiogram

kg = kilogram

KV = kilovolt

min = minute

ml = milliliter

mmHg = millimeters of mercury

mV = millivolt

PRU = blood pressure/blood flow = mmHg/ml/sec

O_2 = oxygen

P = partial pressure

$\dot{Q}$ = blood flow

sec = second

= venous

V_{O_2} = oxygen uptake

2 INTRODUCTION

Homeothermic animals have a reserve of oxygen which will usually last for a few minutes The rapid exchange of respiratory gases is possible because of the specialized pulmonary circulation is totally separated from the systemic circulation in full-grown mammals and birds However the pulmonary circulations in these two groups of animals have developed from different origins In mammals the separating interventricular septum of the heart is the primary septum in the avian heart it is the secondary septum which is also encountered in some reptiles The origin of aortic arches and pulmonary vessels also differ between mammals and birds (Foxon 1955)

Gas exchange in m a m m a l i a n lungs occurs through a thin multilayer membrane Gases move to and from the alveoli when blood flows through the alveolar capillaries Thus a uniform pool model for respiratory gas transfer is formed (Piiper and Scheid 1972) Bronchial arteries supply lung tissues with oxygen-rich blood This vascular system originates from the aorta or its branches and terminates on the right side of the heart Most of the blood in these arteries however perfuses the capillary network that also receives blood from the pulmonary arteries (Daly and Hebb 1966) This blood flows to the left side of the heart through the pulmonary veins and is partly responsible for the so-called physiological shunt (Aviado 1965 Daly and Hebb 1966)

A v i a n species have air sacs that are absent in mammals Avian lungs are fixed into the thorax wall and thus do not collapse when thoracotomy is performed (Burton Besch and Smith 1968) Air flows through the trachea primary bronchi (mesobronchi) and secondary bronchi (dorso- and ventrobronchi see Fig 1) In the region of the tertiary bronchi (parabronchi) the air flow is undirectional from caudal to cranial direction (Hazelhoff 1951 Bouverot and Dejours 1971 Brackenbury 1972 Lasiewski 1972 Scheid Slama and Piiper 1972) The tertiary bronchus air capillaries and atria together form a hexagonal lung unit where the gas exchange occurs (Bremer 1939 Akester 1971 King and Molony 1971 see Fig 2) The gas exchange between

atria and air capillaries takes place by diffusion only (Hazelhoff 1951) The pulmonary arteries bring blood to this lung unit and it is then distributed to the vicinity of the air capillaries by blood capillaries Thus a complex capillary network zone is formed (Fig 3) This network corresponds to the mammalian alveoli Both counter current (Schmidt-Nielsen 1971) and cross current (Piiper and Scheid 1972) principles for gas and blood flows have been proposed Bronchial arteries originate from the aorta carotid arteries or pleural arteries (Owen 1866 Königstein 1903 Westphal 1961) The blood flow through this vasculature as well as the exact organisation of the smallest pulmonary vessels is only poorly known (Akester 1971 b)

The body possesses anatomical chemical and functional reserves in excess of its normal requirements (Kao 1972) At rest acute reduction of the gas exchanging area must and can easily be tolerated In mammals these maximal ventilation-perfusion fluctuations have been discussed by Nordenström (1954) Comroe (1965) and Aviado (1965) They confirm the very rapid normalisation of ventilation and circulation after acute pulmonary artery occlusion

The capability of the avian ventilation and circulation to endure and compensate the acute reductions of the gas exchanging area is far less clearly und[illegible]stood and studied In fact there is no systematic study [illegible] [illegible]rdiorespiratory responses to unilateral pulmonary [illegible] in [illegible] domestic fowl The studies of Bu[illegible] (1968) a[illegible] (1968) will be cited in [illegible]

In mammals chronic [illegible]
ducing a permanent red[illegible]
in the increase of sys[illegible]
lung and in the forma[illegible]
and pulmonary a[illegible]
lung may be 30 per[illegible]
ventricle (Nordenströ[illegible]

The response of the avian lung to the chronic occlusion of one pulmonary artery has been studied by morphological methods only in our laboratory (Länsimies and Karkola 1973) Increases in enzymatic activities (phosphatases esterases amino-peptidases and reductases) suggesting vascular growth and tissue formation were observed Several months after occlusion the enzyme activities describing special functions were somewhat different as compared to the unoccluded state

3 REVIEW OF THE LITERATURE

The avian lung is apparently the most efficient gas exchange apparatus in air breathing vertebrates (Lasiewski and Calder 1971 Weibel 1973) although the effective ventilation/volume ratio is more unequally distributed than in mammals (Scheid and Piiper 1969) The blood flow through the avian lung shows a notably lower $\dot{Q}/\dot{V}O_2$ ratio than the other vertebrates (Johansen 1972)

3 1 Cardiorespiratory parameters of the domestic fowl

The respiration rate of the domestic fowl varies greatly depending on the recording situation Normal values from 20 to 40 breaths per minute have been reported (Piiper et al 1970 see also Table I)

The partial pressures of carbon dioxide and oxygen in the blood of the domestic fowl have previously been determined and are listed in Table I $PaCO_2$ values observed are rather constant 29-30 mmHg Venous PCO_2 is higher about 40 mmHg Lower values have been reported for capillary samples (Bouverot et al 1972) PaO_2 varies from 87 to 110 mmHg venous pressure for oxygen is lower about 40 mmHg Oxygen saturation is 98 per cent when arterial oxygen partial pressure is about 110 mmHg (Burton et al 1968) Arterial pH of unanesthetized domestic fowl is about 7 5 In anesthetized animals lower values have been reported In capillary blood samples the pH is higher

The blood pressure and heart rate of the domestic fowl have been reviewed by Akester (1971 a b) Table II summarizes the studies performed on unanesthetized animals All male and old female animals have a higher blood pressure and lower heart rate than young (4-6 months) female animals (Sturkie Weiss and Ringer 1953 Ringer Weiss and Sturkie 1955 Girard 1973) The role of gonadal hormones is however still obscure (Ringer et al 1957) Body temperature also has an effect on both heart rate and blood pressure (Rodbard and Tolpin 1947) Blood pressure and heart rate vary depending on age and sex The mean systemic arterial blood pressure ranges between 100 and 164 mmHg in young female animals the values reported are close to 140 mmHg Heart rates earlier reported vary within wide limits

from 284 to 378 per minute Young female animals have a heart rate of about 350 per minute

Table III summarizes the results of earlier studies of some hemodynamic parameters of the domestic fowl The cardiac output was first determined by Sapirstein and Hartman (1959) Different methods produce values ranging from 143 ml/kg x min to 270 ml/kg x min Stroke volumes are reported to be between 0 48 and 0 71 ml/kg depending on the age and sex of the animal and the method used Systemic arterial peripheral resistance ranges between 20 and 31 peripheral resistance units (PRU)

Electrocardiology of the avian heart has been reviewed by Jones and Johansen (1972) An avian electrocardiogram was first recorded in 1913 by Buchanan (see Kisch 1951) The P-wave duration is 25 msec PR-interval has been reported to be from 60 81 msec and QRS-duration about 30 msec (Zuckermann 1959 Kisch 1951)

3 2 Cardiorespiratory response to stress in the domestic fowl

The response to h y p o x i a in the domestic fowl is dependent on the degree and duration Butler (1967) and Richards (1971) observed that the respiration rate of intact unanesthetized chickens increases during the initial stages of progressive hypoxia due to rebreathing of respiratory gases During later stages of hypoxia the respiration frequency increases further An intact vagus nerve is essential for an increase in respiration frequency Richards and Sykes (1967) reported that in hens anesthetized with pentobarbitone the respiration rate decreased after an initial increase when progressive hypoxia had reached an arterial pressure of 50 mmHg A similar kind of increase in the respiration rate and ventilation is observed during hypoxia produced by reduction of oxygen in the respiration gas (Ray and Fedde 1969 Bouverot and Leitner 1972) The heart rate increases in the initial phases of hypoxia and then decreases in animals that are awake The initial tachycardia is probably dependent upon autonomic innervation but the final bradycardia is due to a direct anoxic effect in the cardiac tissue (Butler 1967) The same order of changes in cardiac r[illegible] ved in pentobarbitone-

anesthetized animals too (Richards and Sykes 1967) An increase in the heart rate is found to occur only below the arterial PO_2 of 70 mmHg in anesthetized animals (Ray and Fedde 1969) The mean systemic arterial blood pressure during the early stages of hypoxia decreases only slightly (Richards and Sykes 1967) or not at all (Butler 1967) In the later stages of progressive hypoxia there is a marked decrease in blood pressure (Butler 1967 Richards and Sykes 1967 Ray and Fedde 1969) Cardiac output increases during hypoxia due to increases in both heart rate and stroke volume (Butler 1967 Jones and Johansen 1972) The domestic fowl is less tolerant to hypoxia than pigeon or duck (Butler 1970)

Studies concerning the cardiorespiratory response to h y p e r c a p n i a in the domestic fowl have given controversial results The respiration rate has been found either to increase (Leitner 1972) or to decrease (Richards and Sykes 1967 Ray and Fedde 1969) in unanesthetized hypercapnic animals Carbon dioxide sensitive receptors are supposed to be in the lungs of the domestic fowl (Peterson and Fedde 1968 Eaton Fedde and Burger 1971) These receptors are located in the arterial system (Bouverot and Leitner 1972) In anesthetized animals hypercapnia induces a decrease or an increase in respiration rate but an increase in the amplitude of respiration increases the total ventilation This response has been demonstrated in anesthetized tracheal cannulated animals and mechano receptors have been proposed (Bouverot and Leitner 1972) although the presence of pulmonary stretch receptors has been denied by Eaton et al (1971) The ventilatory response to hypercapnia is mediated mainly through the vagus nerve and only partly through the sympathetic nervous system (Burger 1968 Akester and Mann 1969 Cook and King 1969 Fedde 1970) Vagal influence contracting the smooth muscles of the tertiary bronchi controls the ventilation and prevents a CO_2 deficit during hyperventilation (King and Cowie 1969) Heart rate and blood pressure are only slightly modified by moderate hypercapnia (Richards and Sykes 1967) In unanesthetized animals bradycardia and a decrease in cardiac output have been reported (Butler 1967 a b) Ray and Fedde (1969) observed an increase in blood pressure and heart rate during normoxic hypercapnia

in anesthetized animals

A s p h y x i a produces effects similar to or slightly greater than those observed during hypoxia alone The heart rate will fluctuate but bradycardia occurs at the end of the asphyxic period (Richards and Sykes 1967)

Cardiorespiratory response to the o c c l u s i o n o f t h e l e f t p u l m o n a r y a r t e r y in the domestic fowl has been studied in very few and acute situations only The respiration rate was rather high 49 per minute and heart rate rather low 293 per minute in young anesthetized animals with open thorax and occluded left pulmonary artery of unknown period Further no control measurements were made (Rodbard et al 1949) From the results of Peterson and Fedde (1968) and Henry and Fedde (1970) it can be concluded that the respiration rate is increased after unilateral occlusion of both the pulmonary artery and the vein but the sensitivity of the respiration to CO_2 is independent of the blood flow through the lung In these studies the animals were anesthetized and their thoracic and abdominal cavities were opened and they were ventilated by a respirator A slight increase of systemic arterial blood pressure was also observed in their studies Burger (1968) observed that when the content of CO_2 in the ventilation gas was increased from zero to 15 per cent respiration amplitude increased more in animals with an occluded left pulmonary artery than in animals with intact pulmonary circulation The difference was however slight Burton et al (1968) reported a pulmonary arterial hypertension after unilateral occlusion of the pulmonary artery

Enzyme histochemical studies after occlusion of the left pulmonary artery have been performed in our laboratory (Länsimies and Karkola 1973) Immediately after the occlusion nonspecific esterase activity increased in the epithelial cells and the tertiary bronchi Naphthylamidase reaction increased intensely in the epithelium and adjacent structures after the occlusion Capillary network zone showed pronounced tetrazolium reductase activity in a corona like circle around bronchi It was concluded that the active transport mechanisms seem to be disturbed immediately after the occlusion but no

severe ischaemic changes were observed A temporary activation of energy yielding process was noted in the capillary network near the tertiary bronchi A proliferative or inflammatory process was perceptible in the epithelium of the tertiary bronchi and the atrial walls

The d i v i n g p a t t e r n is a universal response to hypoxia produced for example by submerging into water This is a phylogenetically old reflex and all vertebrates seem to respond in a similar manner In natural divers these oxygen saving adjustments operate at a very high degree *of precision (Andersen 1966) The avian response to diving* has been reviewed by Jones and Johansen (1972) In diving animals apnea is promptly followed by bradycardia a redistribution of the regional blood flow and a decrease in cardiac output These changes serve to economize the available oxygen stores (Folkow Nilsson and Yonce 1967 Jones and Johansen 1972) A simulated diving bradycardia in ducks is more dependent upon the progressive hypercapnia than on the increasing anoxia (Feigl and Folkow 1963) Mean arterial blood pressure has been reported to decrease only slightly (Folkow et al 1967 Butler and Jones 1971) although the animals showed a generalized vascular constriction response Myocardial contractility may also be decreased (Folkow et al 1967) although the myocardial circulation is proportionally increased (Johansen 1964) The nervous control of these responses is mainly vagal but evidence of inhibitory sympathetic tone has also been proposed by Folkow et al (1967) An increase in the resistance of the pulmonary outflow and the reduction of the pulmonary artery caliber may be caused by the direct effect of the low alveolar PO_2 or by chemoreceptor reflex (Eliasson 1960 Aakhus and Johansen 1964 Jones and Johansen 1972) The immediate post-dive period results in a rapid normalisation of the observed changes Sometimes this correction is over-shooting and usually of short duration (Folkow et al 1967 Jones and Johansen 1972)

3 3 Morphology of the avian lung

The cross section of a tertiary lung unit varies from 300 to 500 µm the atria from 100 to 200 µm (King and Molony 1971) The blood capillaries are about 7 µm and the air capillaries about 10 µm in diameter The air-blood barrier thickness varies from 0 1 to 0 7 µm and the surface lining of the airways is 0 015 µm in thickness The surface lining is constructed of three layers of 0 005 µm in thickness Two of these layers are osmiophilic and the one between them is non-staining (Petrik and Riedel 1968 a) The diameter of the osmiophilic inclusion bodies varies from 0 5 to 1 0 µm (Jones and Radnor 1972)

Morphology of the gas-exchanging part of the avian lung is schematized in Figures 2 and 3 The small arteries and veins run parallel with the tertiary bronchi The smaller vascular loops carry blood from these small arteries to the veins Air capillary endothelium gradually decreases and becomes indistinct and terminally fuses with the epithelium of the blood capillaries According to Duncker (1972) the blood capillaries originate from septal arterioles (Fig 2) and lead towards the lumen of the tertiary bronchus By the lumen the capillaries converge to form venules and emerge from the tertiary bronchi as small veins Hence it follows that the length of the capillary involved in the gas exchange corresponds approximately to the thickness of one tertiary bronchial lung unit These observations confirm the cross-current exchange principle as described by Scheid and Piiper (1970) In avian lungs the tertiary bronchi are surrounded by a sheath of air capillary/blood capillary meshwork in which gas exchange takes place In this model blood ve sels have gas exchange contact with tertiary bronchial gas inside a single cross-sectional element of the tertiary bronchus only Thus blood flows effectively at a right angle to the air tube axis The arterial blood originates from a mixture of effluent blood whose partial pressure values vary along the length of the air tube (Scheid and Piiper 1970)

Air and blood are separated by a continuous cellular lining (Bremer 1939 Bargmann and Knoop 1961 Nagaishi et al 1964) In electron microscopical studies the osmiophilic membranes have been seen on the epithelial surfaces of the tertiary

bronchi and the atria (Tyler and Pangborn 1964) and air capillaries (Patrik and Riedel 1968 a b) These membranes are probably responsible for the surface tension-reducing properties of the avian lung extracts (Tyler and Pangborn 1964) Osmiophilic laminar inclusion bodies have been found intraepithelially near the site of the surface-active substance production (Adamson and Bowden 1973) as well as in the epithelium of air sacs (Carlson and Beggs 1973) These bodies are seen already during the fetal period (Jones and Radnor 1972 a b) The osmiophilic bodies are probably the side products of surfactant synthesis rather than the immediate source of this substance (Adamson and Bowden 1973) Studies of the innervation of the avian lungs reveal that vagal innervation is dominant (King and Cowie 1969 Akester and Mann 1969 Cook and King 1969 McLelland Cook and King 1972)

4 PURPOSE OF THE PRESENT STUDY

In order to study the adaptativity and efficiency of the circulation and gas exchange in the domestic fowl unilateral pulmonary artery occlusion was performed and the immediate and long term responses were studied by functional and morphological methods:

1 How does the exchange of respiratory gases change after occlusion?

2 What are the hemodynamic responses to occlusion?

3 How does the electrical activity of the heart respond to the changes in circulation after occlusion?

4 How does the blood- air barrier and bronchial vasculature change after occlusion?

5 MATERIAL AND METHODS

A total of 101 white Leghorn chickens were used The animals used in hemodynamic and respiratory studies were young (3 4 months) healthy females In morphological studies the ages at the time of operation and registrations varied more than in cardiorespiratory experiments A full list of animals used in different experiments is given in Tables IV-VI as an appendix to this report The animals used in cardiorespiratory measurements were used in morphological studies too

Water and commercial food were provided ad libitum The cages were indoors 150 x 150 x 90 cm in size Four to six animals were in each cage The temperature was kept constant at 17-18^{o}C The bedding material was raw wood shavings Diurnal rhythm was kept constant by artificial illumination from 7 am to 6 pm

5 1 Operative procedures

An assistant kept the feet and head of the animal gently against the table during operation and registrations The eyes were covered and the animals were lying on their right side The animals were calm during operative procedures Polyethylene tubes 1 mm wide were introduced under local anesthesia of lidocain through the brachial artery and vein into the aorta and anterior caval vein to the level of the right atrium for pressure recordings blood sampling and cardiac output determinations The same tubes and instrumentation were used on all animals The operative method to produce the unilateral pulmonary artery occlusion was modified from Rodbard Brown and Katz (1949) and Burton Besh and Smith (1968) A blunt dissection was made through the left side of the pectoral muscle using local anesthetic The intercostal space was further anesthetized from the angle of the ribs towards the sternum The intercostal space was opened and the ribs parted The left pulmonary artery was easily recognized within a sheath of connective tissue The pulmonary artery was dissected free from the surrounding tissue by a specially designed curved wire hook (10 cm long and the radius of the hook was 4 mm) The artery was pulled laterally (upwards) and closed by surgical

silver clips The intercostal and muscle incisions were closed with silk stay sutures The animals were kept in separate cages for one day postoperatively and then replaced to their original cages The instruments used in the operation were clean but not sterilized

The effect of position on respiration was studied by turning the animal on each side because mammalian respiration is dependent of position (Comroe 1965 Kao 1972)

5 2 Cardiorespiratory recordings

Respiration measurements were performed on 25 young female animals (Table IV) Five of these were controls nine were sham-operated controls and eleven were occlusion-operated Five of the occlusion group were followed for one week and six for two weeks Registrations were carried out before operation three minutes 15 minutes and 30 minutes after occlusion and one or two weeks after operation The thorax was closed between registrations of three and 15 minutes The respiration rate was measured visually during a 30 sec period Arterial and venous blood samples were simultaneously drawn into heparinized glass syringes anaerobically for immediate pH PCO_2 and PO_2 determinations These were performed on IL-227 equipment (Instrumentation Laboratory Milan Italy) at 37^{o}C and corrected for 41^{o}C (Piiper et al 1970) according to the nomogram of Kelman and Nunn (1966) Blood oxygen saturations were measured from the same samples by an oxymeter (Kipp Delft Neatherlands)

Hemodynamic measurements were performed on 39 female animals (Table V) with similar lining as in respiration measurements Ten of the animals served as controls nine were sham-operated controls and 20 were occlusion operated 20 of these five were killed 15 minutes after operation nine were controlled for one week and six for two weeks after occlusion of the left pulmonary artery

Systemic arterial pre sure (mmHg) was registered through a cannula in the aorta Elema pressure transducers calibrated to the level of the heart and a Mingograph 34 recorder (Elema Sweden) were used The mean pre ure was integrated by the

recorder The heart rate was measured from simultaneous ECG recordings or from pressure recordings from periods of 20-30 sec Cardiac output and stroke volume were determined by the indicator dilution method as described by Sturkie and Vogel (1959) and modified by Polkow et al (1967) Indocyanine green (Cardiogreen[R] Hynson and Westcott USA) was injected in small boluses into the anterior caval vein near the right atrium Arterial blood samples were drawn with a pump (Perfusor Braun GFR) through the cannula from the aorta The dye concentrations were determined with Cardiognost apparatus (Atlas Werke GFR) Three to four determinations were performed during each recording period Cardiac output was expressed in ml/min kg b w and stroke volume in ml/kg b w The mean peripheral systemic arterial vascular resistance was calculated by dividing the mean systemic arterial pressure value (mmHg) by the total cardiac output value (ml/sec) This ratio is used as a unit for peripheral vascular resistance (PRU)

Electrocardiographic registrations were performed in 23 occlusion-operated female animals (Table VI) The animals were studied at regular intervals up to six months Needle electrodes were fixed subcutaneously to points 2 cm laterally from the rostral edge of the keel to the right and left sides The third electrode was inserted into the area of the caudal end of the keel These points were considered to correspond the human limb leads and were optimal because if the electrodes were applied under the tight skin of the limbs the animals became restless It is difficult to keep the exact positions of the wings and legs during the long follow-up period In subsequent recordings the localisations were confirmed by puncture scars Registrations were made by Mingograph 34 using bipolar recordings on three channels simultaneously The mean QRS axis was determined from two recordings

5 3 Statistical methods

The method of statistical analysis was the Student s T-test When a comparison is made between two means the p-value is given as a measure for the significance

5 4 Morphological methods

In morphological studies 80 animals were used Immediately after killing following various time intervals from the sham- or occlusion operation the sternum with the keel was removed and occlusion was ascertained as well as the exact location of the tubings Then both lungs were visually examined Tissue samples of 0 5 cubic centimeters were taken from the ventral edge of oth lungs Then the lungs were gently removed from the thorax and macroscopically examined Similar tissue samples were taken from dorsal parts of the lungs These are sites where the tertiary bronchi are most abundant (Duncker 1972) All samples were immediately fixed with neutral or acetic acid-formalinalcohol (Lillie) (Vartia et al 1966) Serial paraffin sections were stained either with hematoxylin eosin using Verhoeff s or Weigert-Domagk s stain A total of more than 1 000 tissue slides were screened microscopically and demonstrative frames were photographed One control and one long term occlusion-operated animal were selected for ultra-microscopical analysis Sixteen tissue samples were immediately fixed with glutaraldehyde solution for six hours then post-fixed with osmium tetroxide for two hours and embedded in epon to be sectioned with Porter-Bloom microtome After sectioning the samples were stained with lead citrate for transmission electronmicroscopy the with toluidine blue for light micro-scopical analysis With this technique the osmiophilic struc-tures survived The sections were examined in a Siemens Elmi-scope I A with an accelerating voltage of 60 kV

In order to visualise bronchial vasculature microangio-graphical studies were made according to the method described by Wendalin and Lindgren (1970) Eight hens were used Three animals were studied 15 minutes after occlusion Five animals including the sham-operated control were studied one week after the operation The animals were heparinized before killing The thorax was immediately opened and the ascending aorta was cannulated just above the pericardium The aorta was then closed at the height of the liver Both ventricles were opened to allow the free flow of the angiographic agent One per cent radiopaque yellow suspension was used as the injection

recorder The heart rate was measured from simultaneous ECG recordings or from pressure recordings from periods of 20-30 sec Cardiac output and stroke volume were determined by the indicator dilution method as described by Sturkie and Vogel (1959) and modified by Folkow et al (1967) Indocyanine green (Cardiogreen[R] Hynson and Westcott USA) was injected in small boluses into the anterior caval vein near the right atrium Arterial blood samples were drawn with a pump (Perfusor Braun GFR) through the cannula from the aorta The dye concentrations were determined with Cardiognost apparatus (Atlas Werke GFR) Three to four determinations were performed during each recording period Cardiac output was expressed in ml/min kg b w and stroke volume in ml/kg b w The mean peripheral systemic arterial vascular resistance was calculated by dividing the mean systemic arterial pressure value (mmHg) by the total cardiac output value (ml/sec) This ratio is used as a unit for peripheral vascular resistance (PRU)

Electrocardiographic registrations were performed in 23 occlusion-operated female animals (Table VI) The animals were studied at regular intervals up to six months Needle electrodes were fixed subcutaneously to points 2 cm laterally from the rostral edge of the keel to the right and left sides The third electrode was inserted into the area of the caudal end of the keel These points were considered to correspond the human limb leads and were optimal because if the electrodes were applied under the tight skin of the limbs the animals became restless It is difficult to keep the exact positions of the wings and legs during the long follow-up period In subsequent recordings the localisations were confirmed by puncture scars Registrations were made by Mingograph 34 using bipolar recordings on three channels simultaneously The mean QRS axis was determined from two recordings

5 3 Statistical methods

The method of statistical analysis was the Student s T-test When a comparison is made between two means the p-value is given as a measure for the significance

6 RESULTS

6 1 Cardiorespiratory response to occlusion of the left pulmonary artery

The results of the respiration studies are given in figures 4 and 5 The respiration rate (Fig 5) of the animals preoperatively was 41 ± 2 breaths per minute in the occlusion-operated group and 40 ± 4 breaths per minute in the sham-operation group The respiration rate increased to 74 ± 5 per minute three minutes after occlusion This increase was significant ($p < 0\ 001$) The significantly higher respiration rate (59 ± 5 $p < 0\ 01$) persisted up to 30 minutes after the occlusion operation and had levelled off to control value during the first postoperation week The sham operation did not alter the respiration rate significantly ($p > 0\ 10$) during the 30 minutes after the manipulation of the pulmonary artery

The arterial PO_2 (Fig 4) in the preoperative situation was 107 ± 2 mmHg and the respective saturation 91 ± 1 % In the sham operation group the respective values were 98 ± 1 mmHg and 97 % Three minutes after occlusion the arterial PO_2 decreased significantly ($p < 0\ 001$) The mean value was 87 ± 4 mmHg and the respective saturation 72 ± 5 % Cyanosis was distinctly visible at this stage in occlusion-operated animals but not in the sham-operated control animals After 15 minutes this hypoxia had vanished and the PaO_2 had returned to 100 ± 1 mmHg This increase from a value of 3 minutes to a value of 15 minutes was significant ($p < 0\ 01$) Oxygen saturation remained significantly lower ($p < 0\ 001$) for 15 minutes after occlusion Venou oxygen tension remained unchanged after occlusion and thus the arterio venous oxygen difference decreased The sham operation did not alter significantly the arterial oxygen tension nor saturation ($p > 0\ 10$) for 30 minutes after the operation The slight decrease in the $P\ O_2$ was significant ($p < 0\ 05$) in contrast to the occlusion-operated group where no change could be observed

Arterial PCO_2 (Fig 5) was 28 ± 1 mmHg in the occlusion-operated group and 22 ± 1 mmHg in the sham-operated controls Occlusion of the left pulmonary artery produced a mild hyper-

capnia $PaCO_2$ was 38 ± 2 mmHg at three minutes and 34 ± 1 mmHg at 30 minutes after occlusion Both these values were significantly above the mean value before operation ($p < 0\ 001$) Arterial hypercapnia levelled off within two weeks following occlusion In the sham-operated control animals $PaCO_2$ showed a slight significant decrease during the 30 minutes after the operation ($p < 0\ 05$) The venous PCO_2 showed a clear increase in occlusion-operated animals

In the control situations before the operation the pH (Fig 5) of arterial and venous blood was 7 51 ± 0 01 and 7 47 ± 0 01 respectively A decrease to 7 42 ± 0 01 and 7 41 ± 0 01 respectively was observed three minutes after occlusion These decreases were statistically significant ($p < 0\ 001$) and remained for 30 minutes but vanished within the first postoperative week In the sham-operated control group the mean pH values were higher than in the occlusion group but did not change significantly ($p > 0\ 10$) after the operation

Turning of the animals on each side for 15 minutes did not change arterial nor venous PO_2 or PCO_2 nor pH The systemic arterial blood pressure as well as heart rate remained essentially unchanged during these procedures

The results of the hemodynamic determinations are given in Figures 6 and 7 Before operation the systemic mean arterial blood pressure (Fig 6) was 152 ± 5 mmHg No significant ($p > 0\ 10$) changes were observed throughout the observation period although a slight trend to increasing blood pressure during 30 minutes and then a decrease to even below the initial control values was observed The sham-operated control animals had essentially unchanged pressure recordings (160 ± 6 mmHg $p > 0\ 10$) during the 30 minutes observation period after the operation

Systemic arterial peripheral vascular resistance (Fig 7) was 11 ± 2 PRU in control situation and decreased to 9 5 fifteen minutes and 6 5 PRU seven days after the occlusion operation The change from the preoperative level to the one week value was almost significant ($p < 0\ 10$) Stroke volume (Fig 7) was 1 7 _ 0 2 ml/beat per kg body weight before the

operation Fifteen minutes after occlusion the mean stroke volume remained unchanged One week after occlusion the stroke volume had increased up to 2 6 ± 0 4 ml/beat/kg b w ($p < 0\ 025$)

Cardiac output was 604 ± 77 ml/min/kg b w in 15 animals before operation In the animals which were used for the occlusion operation the mean cardiac output value before surgical procedures was 661 ± 102 ml/min/kg b w The mean cardiac output 15 minutes after occlusion was essentially the same as before operation One week after occlusion the cardiac output had increased to a mean value of 960 ± 200 ml/min/kg b w ($p < 0\ 10$)

The heart rate of the animals in this study was different in the two experimental groups one for hemodynamic studies and the other for electrocardiographical studies In the hemodynamic group the control value was 317 ± 10 per minute and for sham-operated animals 361 ± 15 per minute The 23 animals of the ECG group had a mean heart rate value of 382 ± 8 per minute before operation The response to the operation was however, similar in both groups The occlusion-operated animals had a progressive bradycardia This bradycardiac response lasted for up to 30 minutes and was significant in both groups ($p < 0\ 02$ and $p < 0\ 001$) The bradycardia normalized within one day, but could be observed in the hemodynamic group even two weeks after occlusion but this late fluctuation in the heart rate was not significant ($p > 0\ 10$) The ham-operated animals of the hemodynamic group showed no variation ($p > 0\ 10$) for 30 minutes after the operation

The results of the electrocardiographical registrations are given in Fig 8 and Table VII The P-wave duration was 19 ± 0 5 msec before operation This changed immediately but only slightly (21 ± 0 7 $p < 0\ 01$) Later after the occlusion operation the P-wave normalized the P-wave amplitude was 0 047 ± 0 007 mV before the operation It increased during the first post-operative day to the value of 0 108 ± 0 006 mV ($p < 0\ 001$) The decrease in the P-wave amplitude was statistically significant ($p < 0\ 01$) from the third post-operative (0 094 ± 0 006 mV) to the two weeks value (0 065 ± 0 005 mV

The PR-interval was 67 ± 1 6 msec before operation There was a slight increase to 80 ± 2 5 msec immediately after the occlusion operation ($p < 0\ 001$) but the interval shortened to the preoperative level during the first day

The QRS-interval was 21 ± 0 8 msec before the operation After occlusion the interval increased up to 23 ± 0 9 msec ($p < 0\ 05$) and remained at a higher level for two weeks after occlusion The mean frontal plane electrical QRS-axis pointed to -67 ± 6^{o} before the occlusion operation indicating a clear left ventricle preponderance The occlusion caused a distinct leftward shift (54 ± 8^{o}) of the axis direction but this was not significant and the QRS-axis had returned to the preoperative direction during the second postoperative week Although they are not indicated in Fig 8 The registrations were performed in 16 animals after three weeks The P-wave duration was 19 msec PR-interval 73 msec QRS-duration 22 msec P-wave amplitude 0 065 mV and the mean electrical axis pointed to -68^{o} In the registration performed during several months of fewer animals no changes could be observed as compared to the 2-3 weeks situation after the occlusion operation

6 2 Morphological response to occlusion

Morphological findings in control animals and in the right lungs of the occlusion-operated ones showed that no changes occurred during the observation period The tertiary bronchioli were 200-500 μm in diameter the atria were 100 to 200 μm in diameter The capillary network zone was 150 to 300 μm in thickness with air capillaries approximately 15 to 20 μm in diameter The blood capillaries never exceeded 20 μm usually the diameter varied between 7 and 10 μm (Fig 9)

Microangiographical studies of both lungs of the control animals and the right lungs of the occlusion-operated animals revealed that the injection of radiopaque substance into the aorta gave only a slight contrast to the capillary network zone The atrial septa were also visualized (Fig 10) and the injected substance came away from the lungs through the pulmonary veins

The tissue between air and blood was very thin 0 3 – 0 6 µm judged from the electron micrographs (Fig 11) The basal membrane between the endothelial cells was 0 12 – 0 15 µm in thickness Small vesicles could be detected on either sides of the basal membrane The air capillaries were regularly covered by a thin osmiophilic lining (Fig 12) The thickness of the lining was about 0 01 µm and it was formed from two osmiophilic membranes with a narrow space between them Osmiophilic inclusion bodies were seen sometimes they were stretched

Immediately after occlusion of the left pulmonary artery the lung resembled pulmonary oedema with enlarged interlobular and interatrial septa and with an increased amount of blood cells in the capillary network zone From the first week onwards the lung was macroscopically firmer as indicated by palpations A profuse arterial blood flowed from the cut of tissue sampling Microangiographic substance stained the occlusion-operated lung bright yellow as compared to the hardly visible colorization of the right lungs of the occlusion-operated animals and both lungs of the control animals In microangiographs (Fig 13 and 14) of the occlusion-operated lungs the pulmonary venules and the capillary network zone were more distinctly radiopaque by the infusate from the aorta through the bronchial arteries as compared to the control lungs. This was seen in the animals already one week after occlusion

After occlusion the thickness of the interlobular septum increased and it contained more connective tissue than the control lungs (Fig 15) The tertiary airways and atria were small and irregular The interatrial muscles were unchanged After occlusion the capillary network zone gradually lost its regular structure and small vessels with greater lumen than in the capillaries were now observed In contrast to the close vicinity of the air and blood in normal lungs these new vessels were distinctly apart from the air capillaries and located in the middle of the capillary network zone The air capillaries were enlarged and the blood capillarie were reduced in number (Fig 16) From four to six months after the operation the occluded lung re embled the mammalian emphysematous lung The interlobular septum was thick and collagenous The interatrial

septum muscles were greatly reduced Air capillaries were large up to 110 µm in diameter The new blood vessels found in the capillary network zone were up to 70 µm in diameter and distinctly apart from air capillaries

Electron micrographs (Fig 17 and 18) of the lung with four months of the post-occlusion period showed that the thickness of the wall between the capillary and blood vessels was 1 to 2 µm and thus many times thicker than in the normal lungs The basal membranes were irregular and had become thick The osmiophilic lining of the air spaces was seen however The intraepithelial osmiophilic inclusion bodies (0 2 - 0 6 µm in diameter) could still be found but they were definitely fewer

7 DISCUSSION

In mammalian lungs the response to unilateral pulmonary artery occlusion is a marked increase in bronchial circulation (Weibel 1960) The increased bronchial circulation to the lung takes part in respiration gas exchange (Bloomer et al 1949) In dogs biochemical effects are quantitative changes in pulmonary phospholipids associated with the production of surfactant but if the post-occlusion period is long no differences were observed by Harlan Margraf and Said (1966) In this laboratory studies of the adaptation to pulmonary artery occlusion with special reference to pulmonary to systemic arterial anastomoses and respiratory distress syndrome have been performed (Thomasson Länsimies and Länsimies 1972) and this study is a part of it

The effects of the operative procedures as such on the registered parameters can be considered minimal as shown in the sham-operated animals Thus this species of animal is suitable for this kind of study where rather massive surgical intervention upon the organism is made The anatomical feature of adhesive pleurae and the easy handling of the domestic fowl further encouraged the use of this avian species in the study of lung physiology The results obtained cannot directly be applied in the mammalian physiology but the repetition of evolution during foetal life makes the thorough understanding of comparative physiological aspects of respiration important

In this study the respiration rate observed in the control situation was slightly higher than in previous studies (Table I) Burton et al (1968) used older animals and Piiper et al (1970) used animals with tracheal cannulation The pronounced tachypnoea of 74 breaths per minute in the occlusion operated animals was normalized during the first post-operative week Whether this tachypnoea increased the actual ventilation was not determined in this study but it is probable according to the studies of Peterson and Fedde (1968) and Bouverot and Leitner (1972) The lack of changes in the sham-operated animals is a further indirect evidence for the physiological significance of the increase in the respiration rate

In the control situation PO_2 and PCO_2 were within the limits given by previous authors (Table I) Occlusion was followed by a short hypoxic period and a visible cyanosis which was not found in the sham-operated animals The hypoxia lasted only a short period as compared to the tachypnoeic period The arteriovenous oxygen difference decreased after occlusion but was unchanced or rather increased after the sham-operation Hypercapnia hyperpnoea and pH decrease appeared immediately after occlusion and persisted longer than hypoxia In sham-operated animals these changes were absent or even opposite to those observed in the occlusion operated animals

Clear homodynamic responses to occlusion were observed The heart rate is under very close autonomic control and tends to vary in different experimental conditions This may explain the large variation in earlier reports (Table II) and the difference observed in this study between the two experimental groups and sham-operated group before operation (Fig 6) The response to occlusion and the absence of change in the sham-operated group however indicates the effect of occlusion The animals with a higher initial heart rate showed a greater decrease in heart rate than the other group with a lower initial heart rate According to Steinschneider and Richmond (1970) the law of initial value explains many of the differences observed in the heart rate responses of for example new born mammals The results of Rodbard et al (1949) obtained in anesthetized animals are in accordance with the results obtained in anesthetized animals are in accordance with the results obtained in this study bradycardia was observed after the occlusion The mean systemic arterial pressure was of the same magnitude as in this study (fig 6) as reported by previous authors (Table II) Significant changes were not observed after occlusion nor after sham-operations

Due to different methods and ages of the animals the cardiac output values registered in the control situation in this study (Fig 7) are considerably higher than those observed in earlier studies (Table III) In ducks using the same method as in the present study comparable values of 250-560 ml/min/kg b w have been reported (Sturkie 1966 Folkow et al 1967

Jones and Holeton 1970) Allometric analysis of resting birds gave a value of 840 ml/min/kg b w for a bird of the size used in the present study (Lasiewski and Calder 1971) The stroke volume value obtained in this study in the control situation (1 7 ml/beat/kg b w) was greater than the values given by previous authors (Table III) but very close to the values (1 37 - 2 45 ml/beat/kg b w) of Sturkie (1966) and Folkow et al (1967) obtained in ducks by the same method as used in this study The occlusion had no immediate effects on cardiac output stroke volume nor systemic arterial peripheral vascular resistance One week after occlusion these parameters had changed uniformly

Acute hypoxia and hypercapnia are both typical features of the diving pattern Domestic fowl is more sensitive to hypoxia than strong flyers and divers (Butler 1970) Thus the response to acute hypoxic and hypercapnic stress may come later and be less accurate than in the natural divers (Andersen 1966) In the presence of ventilation the simultaneous hypoxia and hypercapnia with hyperpnoea and later bradycardia clearly indicate an increased vagal tone The vagus nerve is found to be the main regulator of the diving pattern (Jones and Johansen 1972) The receptors in the avian lungs are chemo- and mechanoreceptors (Eaton et al 1970 Bouverot and Leitner 1972) which both may be involved in the diving response These receptors are mainly in connection with vagal (Akester and Mann 1969) but also with sympathetic nervous systems (Bennet and Malmfors 1970 Fedde 1970) The rapid change in the ventilation perfusion ratio probably through nervous reflectory mechanisms affects the bronchial muscle tone and tends to normalize the ventilation perfusion ratio This mechanism has been found in hypoxic birds by King and Cole (1969) Scheid and Piiper (1970) and Lasiewski (1971) According to Scheid and Piiper (1970) in avian lungs it is possible to have a higher end tidal PCO_2 than in arterial blood In the sham-operated animals on contrary to occlusion operated ones there was a slight decrease in the arterial PCO_2 This may indicate a stre s like action of the sympathetic nervous system The adaptative changes in circulation and the changes in the tone of the autonomic nervous system makes the use of available ventilation and perfusion more effective The determination of the exact role of the vagus nerve will require

direct recordings of the nerve impulses and the blocking of the autonomic receptors in connection with the occlusion

The increases in mean cardiac output and stroke volume values as well as the decrease in peripheral arterial vascular resistance may be considered as compensatory reaction to the reduction of respiratory tissue The decrease in arteriovenous oxygen difference may indicate an increased circulation velocity with a shorter time for oxygen intake and consumption A similar dynamic response as shown in this study during and after loading of the circulation has been observed in other avian species Jones and Holeton (1972) observed increases in cardiac output stroke volume and heart rate in ducks during simulated high altitude hypoxia Feigl et al (1963) and Folkow et al (1967) observed an enormous increase in cardiac output and other parameters during the post dive period in ducks

The effect of occlusion on the electrocardiographic parameters was similar to the changes found in pulmonary embolism The increase in the P-wave amplitude usually called P-pulmonale is a sign of the right atrial overload A temporary and slight slowing down of the conduction velocity PR- and QRS-intervals confirmed this enlargement of the heart A clearcut deviation of the frontal plane QRS-axis to the left also was suggestive of the analogy to pulmonary embolism In man and dog occlusion of the pulmonary artery is most often followed by a left-ward shift of the QRS-axis (Zuckermann et al 1950 Lynch Stein and Bruce 1972) In the present study the shrinkage and atrophy of the left lung (possibly resulting in a better electrical conductivity) and hemodynamic acceleration may explain the changes observed in the electrocardiogram

In mammals the occlusion operation has induced similar changes in respiration and circulation as observed in this study in the domestic fowl In man a temporary occlusion of the pulmonary artery produces a slight hypoxia and hypercapnia (Brofman et al 19 7) Rosencrantz et al (1973) observed a 50 per cent increase in cardiac output after a 2-4 weeks occlusion period Pulmonary hypertension increase in the pulmonary resistance and hypoxia were observed after the chronic occl[illegible] pigs

The morphology and morphometric featur[illegible] normal lungs

of the domestic fowl in this study were similar to those reported e g by Weibel (1973) Osmiophilic inclusion bodies and surface lining have been found in the lungs of mammals (Kikkawa Motoyama and Gluck 1968) reptiles (Dierichs 1973) and lungfishes (Weibel 1973) In mammals these lamellar structures have been related to the production and transfer of the surfactant and the same has been proposed in avian species too (Tyler and Pangborn 1964) This has been opposed however by Jones and Radnor (1972 b) The morphological response to occlusion was a gradual loss of regular features The distance from the air to the blood capillary increased Osmiophilic lamellar inclusions decreased in number but were found The surface membrane of the air capillary survived

The infusion of the radiopaque substance to the aorta revealed that the main route for outflow of the bronchial blood is the pulmonary venous system This bronchial vasculature is scarce in normal state One week after occlusion a distinct increase in the systemic vascularity was observed These vessels drained through the pulmonary veins Thus the bronchial vasculature in the domestic fowl drains like in mammals and the response to pulmonary artery occlusion is similar to the mammalian response

By histochemical methods Tyler and Pearse (1966) and Länsimies and Karkola (1973) have found the highest enzyme activities near the tertiary bronchi and the atria Alkaline phosphatase and non-specific esterase reactions were very clear These enzymes synthetize the surface active phospholipids The enzymatic activities in the lung after occlusion operation first decrease and then increase during the active proliferative phase of growth of bronchial vasculature during the first postoperative weeks (Länsimies and Karkola 1973) The activity of naphthylamidase reaction indicated proliferative connective tissue during first two weeks after the occlusion The activity of the succinate tetrazolium reductase indicates an altered metabolism in the lung with pulmonary artery occlusion and increased bronchial arterial flow

The respiratory circulatory and morphological response to the occlusion of the left pulmonary artery can be divided into three phases The first acute stage with the reduction of the gas exchanging capacity is tolerated mainly by a reflectory process resembling the dividing pattern This response consisted of bradycardia tachypnoea and probably bronchoconstriction

The second stage the beginning of adaptation is mainly functional Circulatory changes have the main part although the adaptative changes in the blood cells are possible At this stage the respiratory gas tensions normalize and the heart rate returns to control level the contractile efficiency of the heart increases as shown by the increased stroke volume and cardiac output and changes in the electrocardiogram

During the this stage of adaptation morphological reorganisation the bronchial vasculature increases enzyme activities become restabilized (Länsimies and Karkola 1973) and osmiophilic substances are found Thus at least other functions of the lung than only respiration (temperature regulation water evaporation etc) are possible (Lasiewski 1972) Whether this bronchial circulation is capable of gas exchange as in mammals and what are the hematological adaptative features remain to be seen in the further studies

8 ABSTRACT

In order to study the capability of the gas exchange function and blood circulation to tolerate and adapt to the occlusion of the left pulmonary artery in the domestic fowl occlusion operations were performed in a series of 101 animals The respiration rate arterial and venous oxygen and carbon dioxide partial pressures and oxygen saturation arterial blood pressure heart rate cardiac output stroke volume peripheral vascular resistance and electrocardiogram were registered Morphology of the lungs was studied by histological microangiographical and electronmicroscopical methods

The response could be divided in to three stages An acute response to the occlusion (from minutes to hours) tachypnoea with initial hypoxia and hypercapnia and bradycardia were found This response resembled the diving pattern dominated by the increased vagal tone Bradycardia and hypoxia returned to the control values in one day Tachypnoea and elevated $PaCO_2$ were still evident after one week

During the second stage functional adaptation (from days to weeks) increases in cardiac output and stroke volume could be a sign of compensatory enhancement of the circulation and thus considered as induced adaptation process Electrocardiographic changes increased P-wave amplitude and QRS-duration further pointed to the temporary increased load of the right side of the heart and later to the improved effectivity of the left ventricle

The third stage morphological reorganisation (from week onwards) partly explains the increased output of the heart The increase in the bronchial vasculature probably also indicated increased flow The run-off of this new vasculature in the gas exchange zone was through pulmonary veins In addition the osmiophilic structures and the epithelial surfactant were only slightly reduced These morphological observations together with enzyme histochemical studies previously reported by the author indicate that the occluded lung still had largely its functional abilities

9 ACKNOWLEDGEMENTS

The present study was performed 1970-1974 at the Department of Physiology and Cardiorespiratory Research Unit University of Turku

I wish to express my profound thanks to Professor Kaarlo Hartiala Rector Magnificus of my University and the Head of the Department of Physiology for his interest towards my efforts and the great work he is doing for the benefit of free science

My sincere gratitude is indebted to Professors Leo Hirvonen University of Oulu and Tuomas Peltonen University of Turku for initiation of and guidance in this study They are the founders and Heads of the Cardiorespiratory Research Unit Their vast knowledge of the experimental physiology of circulation and respiration has been of paramount importance for me Without their kind help my work would have been unsuccessful

I want to thank associate Professor Hannu Suomalainen University of Turku and Doc Pekka Putkonen University of Helsinki for their thorough and sound criticism and help during the preparation of the manuscript Furthermore I would like to thank Professor Suomalainen for permission to use the facilities of his department

I wish to express my gratitude to Professor Osmo Järvi Head of the Department of Pathological Anatomy University of Turku for permission to use the facilities of his department Also his kind attitude towards my studies of the morphology of avian lungs is greatly acknowledged

Associate Professor Pentti Valleala was the acting Head of the Department of Physiology University of Turku during the time of my study This difficult task he performed with admirable patience

Associate Professor Rauno Tirri was a real fried guiding me through the deepest depressions The afternoons I spent with him were essential for the final version of this study

To all my colleagues and friends from the Cardiorespiratory Research Unit and the Department of Physiology I express my gratitude Especially Associate Professor Ilkka Välimäki Doc Heikki Wendelin Dr Antero Aitio and MSc Lauri Halkola have been of great help during this study

My gratitude is indebted to Professor John Typpo and Ms Janice Kaartinen for revising the English of my manuscript They had a difficult job and all the possible faults are due to my obstinacy

Ms Aili Ryynänen MSc and her associates in the Library have been very helpful during the years of this study

The first ladies of the departments Ms Kerttu Eeva Loiske and Ms Irma Salminen have performed most of the practical work that is so important for this kind of study My moods they tolerated with patience During the study Mr Unto Porttinen and Mr Urho Lehtimäki helped me in many ways thank you

Ms Hellin Aaltonen and Ms Marketta Kiviniitty typed the manuscript over and over again thank you

I recieved two scholarships from the Finnish Academy of Science they were important for me

Finally I would like to express my apologies to my family My ups and downs (mostly downs) have been difficult to tolerate Now there is time for us

Naantali December 1974

Esko Länsimies

PS During the preparation of the manuscript the centenary anniversary of the birth of August Krogh (15 11 1874 - 14 9 1949) took place His life-long work on the comparative physiology of respiration is monumental and would deserve a lot more respect than just mention in this connection

REFERENCES

AAKHUS T and K JOHANSEN Angiography of the duck during submersion asphyxia Acta physiol scand 1964 63 10–17

ADAMSON I Y R and D H BOWDEN The intracellular site of surfactant synthesis Autoradiographic studies on murine and avian lung explants Exp Molec Pathol 1973 18 112–124

AKESTER A R The heart In: D J Bell and B M Freeman: physiology and biochemistry of the domestic fowl Vol 2 Acad Press London 1971 a

AKESTER A R The blood vascular system In: D J Bell and B M Freeman: Physiology and biochemistry of the domestic fowl Acad Press London 1971 b

AKESTER A R and S P MANN Ultrastructure and innervation of the tetriary-bronchial unit in the lung of Gallus Domesticus J Anat 1969 105 202–204

ANDERSEN H T Physiological adaptations in diving vertebrates Physiol Rev 1966 46 212–241

AVIADO D A The bronchial circulation In: The lung circulation Vol 1 Pergamon Press New York 1965

BARGMAN W and A KNOOP Electronenmikroskopische Untersuchungen an der Reptilien-und Vogellunge Z Zellforsch 1961 54 541–548

BENNET T and T Malmfors The adrenergic nervous system of the domestic fowl Z Zellforsch 1970 106 22–50

BLOOMER W E W HARRISON G E LINDSKOG and A A LIEBOW Respiratory function and blood flow in the bronchial artery after ligation of the pulmonary artery Amer J Physiol 1949 157 317–328

BOUVEROT P and P DEJOURS Pathway of respired gas in the air sacs-lung apparatus of fowl and ducks Respiration Physiol 1971 13 330–342

BOUVEROT P and L -M LEITNER Arterial chemoreceptors in the domestic fowl Re piration Physiol 1972 15 310–320

BRACKENBURY J H Physical determinants of air flow pattern within the avian lung Respiration Physiol 1972 15 384–397

BREMER J L Evidence of an epithelial lining in the labyrinth of the avian lung Anat Rec 1939 73 497-513

BROFMAN B L B L CHARMS P M KOHN J ELDER R NEWMAN and M RIZIKA Unilateral pulmonary artery acclusion in man J Thoracic Surg 1957 34 204-227

BURGER R E Pulmonary chemosensitivity in the domestic fowl Fed Proc 1968 27 328

BURTON R R E L BESCH and A H SMITH Effect of chronic hypoxia on the pulmonary arterial blood pressure of the chicken Am J Physiol 1968 214 1438-1442

BUTLER P J The effect of progressive hypoxia on the respiratory and cardiovascular systems of the chicken J Physiol 1967 191 309-324

BUTLER P J The effect of progressive hypoxia on the respiratory and cardiovascular systems of the pigeon and duck J Physiol 1970 201 527-538

BUTLER P J and D R JONES The effect of variations in heart rate and regional distribution of blood flow on the normal pressor response to diving in ducks J Physiol 1971 214 457-479

CARLSON H C and E C BEGGS Ultrastructure of the abdominal air sac of the fowl Res vet Sci 1973 14 148-150

CHAPOT G N BARRAULT M MULLER and N DARGNAT Comparative study of P_{aCO_2} in several homeothermic species Am J Physiol 1972 223 1354-1357

CHIODI H and J W TERMAN Arterial blood gases of the domestic hen Am J Physiol 1965 208 798-800

COMROE J H Physiology of respiration Year Book Medical Publishers Chicago 1965

COOK R D and A S KING Nerves of the avian lung: electron microscopy J Anat 1960 105 202

DALY I De B and C O HEBB Pulmonary and bronchial vascular systems Edward Arnold Publishers Great Britain 1966

DIERICHS R Elektronenmikroskopische Untersuchungen an der Froschlunge I Darstellung der Alveolar-grenzschicht Z Zellforsch 1973 137 553-561

DUNCKER H R Structure of avian lungs Respiration Physiol 1972 14 44-63

EATON J A Jr M R FEDDE and R E BURGER Sensitivity to inflation of the respiratory system in the chicken Respiration Physiol 1971 11 167-177

FEDDE M R Peripheral control of avian respiration Fed Proc 1970 29 1664-1673

FEIGL E and B FOLKOW Cardiovascular response in diving and during brain stimulation in ducks Acta physiol scand 1963 57 99-110

FOLKOW B N J NILSSON and L R YONCE Effects of diving on the cardiac output in ducks Acta physiol scand 1967 70 347-361

FOXON G E H Problems of the double circulation in vertebrates Biol Rev 1955 30 196-228

GIRARD H Arterial pressure in the chick Am J Physiol 1970 224 454-460

HARLAN W R Jr J H MARGHAP and S I SAID Pulmonary lipid composition of species with and without surfactant Am J Physiol 1966 211 855-861

HAZELHOFF E H Structure and function of the lungs of birds Poultry Sci 1951 30 3-10

HENRY J D and M R FEDDE Pulmonary circulation time in the chicken Poultry Sci 1970 49 1286-1290

JOHANSEN K Regional distribution of circulating blood during submersion asphyxia in the duck Acta physiol scand 1964 62 1-9

JOHANSEN K Heart and circulation in gill skin and lung breathing Respiration Physiol 1972 14 193-210

JONES A W and A J BARSON Elastogenesis in the developing chick lung a light and electron microscopical study J Anat 1971 110 1-15

JONES A W and J P RADNOR The development of the chick tertiary bronchus I General development and the mode of production of the osmiophilic inclusion body J Anat 1972 a 113 303-324

JONES A W and J P RADNOR The develpment of the chick tertiary bronchus II The origin of the surface lining system J Anat 1972 b 113 325-340

JONES D R and G F HOLETON Cardiovascular and respiratory response of ducks to progressive hypocapnic hypoxia J Exp Biol 1972 56 657-666

JONES D R and K JOHANSEN The blood vascular system of birds In: D S Farner and J R King: Avian biology vol II 157-285 Acad Press London 1972

KAO F F Respiration physiology Excerpta Medica Amsterdam 1972

KELMAN G R and J F NUNN Nomograms for correction of blood P_{O_2} P_{CO_2} pH and base excess for time and temperature J appl physiol 1966 21 1484-1490

KIKKAWA Y E K MOTOYAMA and L GLUCK Study of the lungs of fetal and newborn rabbits Morphological biochemical and surface physical development Am J Pathol 1968 52 177-210

KING A S and A F COWIE The functional anatomy of the bronchial muscle of the bird J Anat 1969 105 323-336

KING A S and V MOLONY The anatomy of respiration In: D J Bell and B M Freeman: Physiology and biochemistry of the domestic fowl vol 1 Acad Press London 1971

KISCH B The electrocardiogram of birds (chicken duck pigeon) Exp Med Surg 1951 7 103-124

KONIGSTEIN H Zur Morphologie und Physiologie des Gefässsystems am Respirationstrakt Anatomische Hefte 1903 22 309-375

LÄNSIMIES E and K KARKOLA Enzyme histochemical studies of the lungs of domestic fowl Acta path microbiol scand Sec A 1973 81 159-166

LASIEWSKI R C Respiratory function in birds In: D S Faner and J R King: Avian Biology II Acad Press London 1972

LASIEWSKI R C and W A CALDER Jr A preliminary allometric analysis of respiratory variables in resting birds Respiration Physiol 1971 11 152-166

LEITNER L M Pulmonary mechanoreceptor fibers in the vagus of the domestic fowl Respiration Physiol 1972 16 232-244

LYNCH R E P D STEIN and T A BRUCE Leftward shift of frontal plane QRS-axix as a frequent manifestation of acute pulmonary embolism Chest 1972 61 443-446

McLelland J R D COOK and A S KING Nerves in the exchange area of the avian lung Acta anat 1972 83 7-16

NAGAISHI C H Y OKADA S ISHIKO and S DAIDO Electron microscopic observations of the pulmonary alveoli Exp Med Surg 1964 22 81-117
NORDENSTRÖM B Temporary unilateral occlusion of the pulmonary artery Acta radiol Suppl 108 1954
OSHIMA S K SHIMADA and T TONOVE Radio telemetric observations of the diurnal changes in respiration rate heart rate and intestinal motility of domestic fowl Poultry Sci 1974 53 503-507
OWEN R Circulating system of birds In Anatomy of vertebrates vol II Longmans Green and Co London 1866
PETERSON D F and M R FEDDE Receptors sensitive to CO_2 in lungs of chicken Science 1968 162 1499-1501
PETRIK P and B RIEDEL An osmiophilic bilaminar lining film at the respiratory surfaces of avian lungs Z Zellforsch 1968 a 88 204-219
PETRIK P and B RIEDEL A continuous osmiophilic noncellular membrane at the respiratory surface of the lungs of fetal chickens and of young chicks Lab Invest 1968 b 18 54-62
PIIPER J F DREES and P SCHEID Gas exchange in the domestic fowl during spontaneous breathing and artificial ventilation Respiration Physiol 1970 9 234-249
PIIPER J and P SCHEID Maximum gas transfer efficacy of models for fish gills avian lungs and mammalian lungs Respiration Physiol. 1972 14 115-124
RAY P J and M R FEDDE Responses to alteration in respiratory P_{O_2} and P_{CO_2} *in the chicken* Respiration Physiol 1969 6 135-143
RICHARDS S A Brain stem control of polypnoea in the chicken and pigeon Respiration Physiol 1971 11 315-326
RICHARDS S A and A H SYKES The effects of hypoxia hypercapnia and asphyxia in the domestic fowl Comp Biochem Physiol 1967 21 691-701

RINGER R K H S WEISS and P D STURKIE Effect of sex and age on blood pressure in the duck and pigeon Am J Physiol 1955 183 141-143

RINGER R K H S WEISS and P D STURKIE Heart rate of chickens as influenced by age and gonadal hormones Am J Physiol 1957 191 145-147

RODBARD S F BROWN and L N KATZ The pulmonary arterial pressure Am Heart J 1949 38 863-871

RODBARD S and D FELDMAN Relationship between body temperature and arterial pressure Proc Soc Exp Biol Med 1946 63 43-50

RODBARD S and M TOLPIN A relationship between the body temperature and the blood pressure in the chicken Am J Physiol 1947 151 509-515

ROSENCRANTZ J G J H CARLISLE F P LYNCH and J H K VOGEL Ligation of a single pulmonary artery in the pig: a model of chronic pulmonary hypertension J Surgical Res 1973 15 67-73

SALT G W and E Zeuthen The respiratory system In: Marshall A J : Biology and comparative physiology of birds vol 1 Acad Press New York 1960

SAPIRSTEIN L A and F A HARTMAN Cardiac output and its distribution in the chicken Am J Physiol 1959 196 751-752

SCHEID P and J PIIPER, Volume ventilation and compliance of the respiratory system in the domestic fowl Respiration Physiol 1969 6 298-308

SCHEID P and J PIIPER Analysis of gas exchange in the avian lung Theory and experiments in the domestic fowl Respiration Physiol 1970 9 246-262

SCHEID P and J PIIPER Cross current gas exchange in avian lungs: Effects of reversed parabroncial air flow in ducks Respiration Physiol 1972 16 304-312

SCHEID P H SLAMA and J PIIPER Mechanisms of unidirectional flow in parabronchi of avian lungs: measurements in duck lung preparation Respiration Physiol 1972 14 83-95

SCHMIDT-NIELSEN K How birds breathe? Scientific Amer 1971 225 72-79

STEINSCHNEIDER A and J B RICHMOND The autonomic nervous system In: U Stave Physiology of the neonatal period Appleton-Century-Crofts New York 1970

STURKIE P D Cardiac output in ducks Proc Soc exp Biol Med 1966 123 487-488

STURKIE P D Cardiovascular effects of acclimatization to heat and cold in chickens J appl Physiol 1967 22 13-15

STURKIE P D Y C LIN and N OSSORIO Effects of acclimatization to heat and cold on heart rate in chickens Am J Physiol 1970 219 34-36

STURKIE P D and J A VOGEL Cardiac output central blood volume and peripheral resistance in chickens Am J Physiol 1959 197 1165-1166

STURKIE P D H S WEISS and R.K RINGER Effects of age on blood pressure in the chicken Am J Physiol 1953 174 405-407

THOMAS S P and R A SUTHERS The physiology and energetics of bat flight J Exp Biol 1972 57 317-335

THOMASSON B E LÄNSIMIES and H LÄNSIMIES Intrauterine ligation of the left pulmonary artery in the rabbit Acta Pharmacol Toxicol 1972 31 suppl 1 131

TYLER W S and J PANGBORN Laminated membrane surface and osmiophilic inclusions in avian lung epithelium J Cell Biol 1964 20 157-164

TYLER W S and A G E PEARSE Functional and analytical hi tochemistry of the chicken lung lobule with particular reference to surfactant Poultry Sci 1966 45 501-511

VARTIA A P TERHO H SUOMALAINEN T PELTONEN and L HIRVONEN Structural and functional development of the chick lung from second week of incubation until hatching Acta physiol scand 1966 68 suppl 227 211

WEIBEL E R Early stages in the development of collateral circulation to the lung in the rat Circul Res 1960 8 353-376

WEIBEL E R Morphological basis of alveolar-capillary gas exchange Physiol Rev 1973 53 419-495

WENDELIN H and I LINDGREN Microangiography of the renal vessels in rabbit Comparison of different methods Acta Radiol (Diagn) (Stockh) 1970 10 49-56

WESTPHAL U Das arteriensystem des Haushuhnes Wiss Z Humboldt-Univ Berlin Math -Nat R 1961 X 93-124

VOGEL J A and P D STURKIE Cardiovascular responses of the chicken to seasonal and induced temperature changes Science 1963 140 1404-1406

WOODBURY R A and W F HAMILTON Blood pressure studies in small animals Am J Physiol 1937 119 663-674

ZUCKERMANN P Vögel-EKG In: Grundriss und Atlas der Elektrokardiographie G Thieme Leipzig 1959

ZUCKERMANN R M J RODRIQUEZ D SODI-PALLARES and A BISTENI Electropathology of acute cor pulmonale Am Heart J 1950 40 805-824

11 APPENDIX

Tables II - VIII
Figures IX - XXV

TABLE I Respiration rate P_{O_2} P_{CO_2} and pH of the blood of the domestic fowl

Reference	Year	Respiration rate breaths/min	pH	P_{CO_2} mmHg	P_{O_2} mmHg	Notes
Chiodi et al	1965	-	7 49	32 8	99 1	arterial
Richards et al	1967	27	7 47	-	95	anesthesia arterial
Burton et al	1968	26 4	7 51	34 6	109 9	male arterial
- -	-	31 0	-	-	-	female arterial
Piiper et al	1970	23	-	29 2	87 0	arterial
- -	- -	23	-	39 3	40 8	venous
Bouverot et al	1972	-	7 55	24 5	89 5	capillary
Oshima et al	1974	38 5	-		-	male

TABLE II Blood pressure and heart rate in the domestic fowl

Reference	Year	Mean arterial pressure mmHg	Heart rate beats/min	Age	Sex
Woodbury et al	1937	161	-	?	?
Rodbard et al	1946	100	-	?	?
Rodbard et al	1947	125	-	Young	?
Sturkie et al	1953	131	328	Fullgrown	Female
Sturkie et _l	1953	164	264	Fullgrown	Male
Ringer et al	1957	-	326	Young	Female
Ringer et al	1957	-	286	Young	Male
Sturkie et al	1959	142	378	Young	Female
Sturkie et al	1959	166	307	Young	Male
Zuckerman	1959	-	353	?	?
Vogel _t al	1963	144	356	?	Female
Sturkie	1967	168	-	Fullgrown	Male
Burton et al	1968	-	350	?	Female
Sturkie et al	1970	-	303	?	Male
Piiper et al	1970	144	377	Young	Female
Oshima et al	1974	-	332	Young	Male

TABLE III Cardiac output stroke volume and peripheral arterial resistance in the domestic fowl

Reference	Year	Cardiac output ml/min/kg	Stroke volume ml/beat/kg	Peripheral resistance mmHg / ml/sec	Method	Anesthesia	Sex
Sapirstein et al	1959	218	-	-	86Rb-dilution	Pento-barbitone	?
Sturkie et al	1959	143	0 49	31	Dye dilution	-	male
	- -	173	0 46	29	- -	-	female
Vogel et al	1963	240	0 67	22	- -	-	- -
Sturkie	1967	180	-	21	- -	-	male
Piiper et al	1970	270	0 71	20	- -	-	female

TABLE IV List of the 3-4 months old female animals used in the respiration studies The identification number and weight (kg) of the animals are indicated Recordings were made before and after the occlusion of the left pulmonary artery

	No	Weight	Control	3	15	30	1 week	2 weeks
Control animals	75	1 6	x					
	76	1 6	x					
	78	1 5	x					
	81	1 7	x					
	87	1 7	x					
Sham-operated controls	93	1 0	x	x	x	x		
	94	1 1	x	x	x	x		
	95	1 2	x	x	x	x		
	96	1 2	x	x	x	x		
	97	1 1	x	x	x	x		
	98	1 2	x	x	x	x		
	99	1 0	x	x	x	x		
	100	1 2	x	x	x	x		
	101	1 3	x	x	x	x		
Examined for one week after occlusion	77	1 6	x	x	x	x	x	
	84	1 7	x	x	x	x	x	
	88	1 7	x	x	x	x	x	
	89	1 4	x	x	x	x	x	
	91	1 7	x	x	x	x	x	
Examined for two weeks after occlu ion	79	1 7	x	x	x	x		x
	80	1 7	x	x	x	x		x
	82	1 7	x	x	x	x		x
	85	1 7	x	x	x	x		x
	86	1 7	x	x	x	x		x
	90	1 7	x	x	x	x		x

TABLE V List of the 3-4 months old female animals usod in the hemodynamic studies The identification number and weight (kg) are indicated

	No	weight	Control	3	15	30	1 week	2 we
Control	1	1 7	x					
animals	17	1 6	x					
	65	1 9	x					
	66	1 8	x					
	74	1 5	x					
	75	1 6	x					
	76	1 6	x					
	78	1 5	x					
	81	1 7	x					
	87	1 7	x					
Sham-	93	1 0	x	x	x	x		
operated	94	1 1	x	x	x	x		
controls	95	1 2	x	x	x	x		
	96	1 2	x	x	x	x		
	97	1 1	x	x	x	x		
	98	1 2	x	x	x	x		
	99	1 0	x	x	x	x		
	100	1 2	x	x	x	x		
	101	1 3	x	x	x	x		
Examined	10	1 5	x		x			
15 min	67	1 5	x		x			
after	68	1 3	x		x			
occlusion	69	1 2	x		x			
	70	1 5	x		x			
Examined	64	1 3	x		x		x	
for one	71	1 4	x		x		x	
week after	72	1 5	x		x		x	
occlusion	73	1 4	x		x		x	
	77	1 6	x	x	x	x	x	
	84	1 7	x	x	x	x	x	
	88	1 7	x	x	x	x	x	
	89	1 4	x	x	x	x	x	
	91	1 7	x	x	x	x	x	
Examined	79	1 7	x	x	x	x		x
for two	80	1 7	x	x	x	x		x
weeks	82	1 7	x	x	x	x		x
after	85	1 7	x	x	x	x		x
occlusion	86	1 7	x	x	x	x		x
	90	1 7	x	x	x	x		x

TABLE VI List of the occlusion operated animals used in the electrocardiographical recordings Identification number and weight (kg) of the animal is indicated Registrations were performed before and after occlusion of the left pulmonary artery

No	Weight	Control	15	1-2 days	3 days	1 week	2 weeks
21	0 8	x	x				
27	0 9	x	x	x	x	x	
28	1 0	x	x	x	x	x	x
29	0 8	x	x	x	x		
31	1 7	x	x	x	x	x	x
33	1 7	x	x	x	x	x	x
36	2 0	x	x	x	x	x	x
39	1 8	x	x	x	x	x	x
40	1 8	x	x	x	x	x	x
41	2 0	x	x	x	x	x	x
42	2 0	x	x	x	x		
43	2 2	x	x	x	x	x	x
44	2 3	x	x	x	x	x	x
45	2 3	x	x	x	x	x	x
46	2 0	x	x	x	x	x	x
49	2 5	x	x	x	x	x	x
50	1 5	x	x	x	x	x	x
51	1 5	x	x	x	x	x	x
52	1 6	x	x	x	x	x	x
53	2 5	x	x	x	x	x	x
54	1 8	x	x	x	x	x	x
55	2 4	x	x	x	x	x	x
56	2 5	x	x	x	x	x	x

TABLE VII Electrocardiographical parameters (means in the domestic fowl before and after occlusion of the left pulmonary artery

	Control	15	1 day	3 days	1 week	2 weeks
P-wave duration (msec)	19	21	20	20	20	19
PR-interval (msec)	67	80	69	72	72	70
QRS-duration (msec)	21	23	23	23	23	23
P-wave amplitude (mV)	0 047	0 062	0 108	0 094	0 073	0 065
Mean electrical axis (degrees)	-67	-54	-56	-55	-61	-72

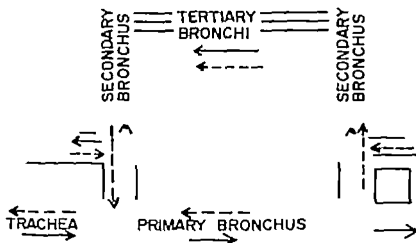


Fig 1 Direction of the flow of air in the avian lung during spontaneous inspiration (——▸) and expiration (----▸)

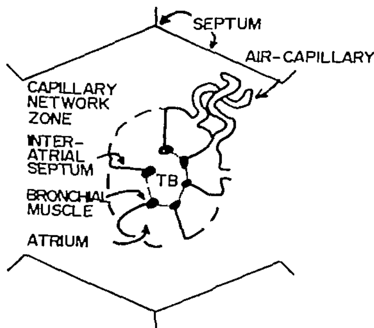


Fig. 2 Cross section of a tertiary unit TB = tertiary bronchus

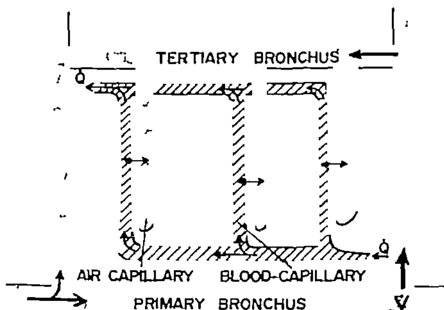
x

FIGURE 3

Scheme of the gas-exchanging apparatus of the avian lung modified from Salt and Zeuthen (1960) and Scheid and Piiper (1970) Arrows show the direction of blood and air

FIGURE 4

P_{O_2} in mmHg (•) and blood oxygen saturation in per cents (o) in arterial (——) and venous (--) blood (Mean ± SEM) before and after unilateral occlusion of the pulmonary artery or sham operation (-) in the domestic fowl



mmHg %

100—100

50—50

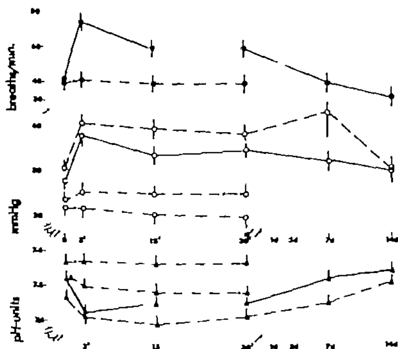
0 5' 15 30' 1d 3d 7d 14d

FIGURE 5

Respiration rate in breaths per minute (●) P_{CO_2} in mmHg (o) and pH (Δ) in arterial (——) and venous (---) blood (mean ± SEM) before and after occlusion of the left pulmonary artery or sham operation (_:) in the domestic fowl

FIGURE 6

Mean arterial pressure in mmHg (●) and heart rate in beats per minute in ECG-group (Δ) and hemodynamic group (o) sham-operated animals are indicated by a dotted line (- -)


breaths/min.
mmHg
pH-units

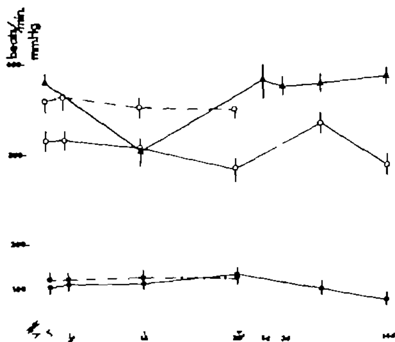
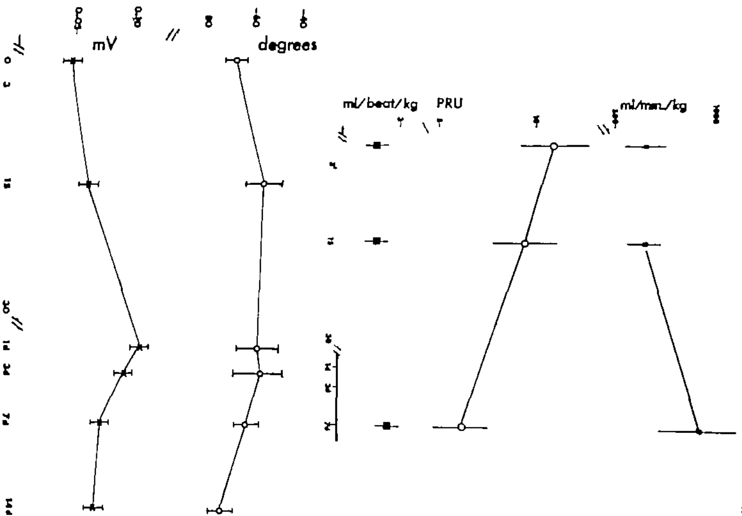

beats/min.
mmHg

FIGURE 7

Arterial peripheral vascular resistance in PRU (o) cardiac output in ml/min/kg b w (•) stroke volume (□) in ml/beat per kg b w (mean ± SEM) before and after unilateral occlusion of the pulmonary artery in the domestic fowl

FIGURE 8

Mean electrical axis in degrees (o) and P-wave amplitude in mV (x) (mean ± SEM) before and after unilateral occlusion of the pulmonary artery in the domestic fowl



mV
0.05
0.10
degrees
-40
-60
80
ml/beat/kg
PRU
ml/min./kg
1000
0
3
15
30
1d
3d
7d
14d
XV

FIGURE 9

Left lung of a young control animal Verhoeff s elastic staining The scale is indicated in the figure (50 /um) The tertiary bronchus and atria are seen on the left side of the figure Smooth muscle ring has a diameter of 250 /um Some of the atria are visible as open some as collapsed spaces Elastic fibers are seen in the inter-atrial walls In the middle of the figure a septum is seen with two arteries diameters about 70 /um Light microscopy control lung

FIGURE 10

Right lung of the occlusion-operated young animal The scale is indicated in the figure (100 /um) Several hexagonal lunglobuli are seen a poor filling of the septa a rather good filling of the inter-atrial walls (arrow) and the capillary network zone Microangiograph control lung injection through the aorta

FIGURE 11

Young control animal the scale is indicated in the figure (0 5 μm) A blood capillary with a rod cell (BC) and an air capillary (AC) are visible The basal membrane (asterisk) is regular and thin Osmiophilic lamellar structures are shown with an arrow The thin surface lining of the air capillary is indicated with small arrows
Electron micrograph control lung

FIGURE 12

Explanations as in Fig 9 note the dual osmiophilic lamina on the air-capillary surface (small arrows)
Electron micrograph control lung

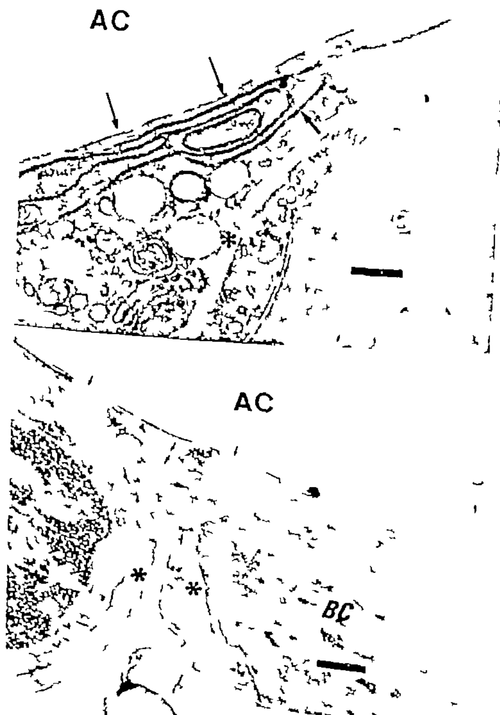

AC
*
AC
*
*
BC

FIGURE 13

Left lung of a young female chicken occlusion-operated one week earlier The scale is indicated in the figure (100 /um) Centrifugal distribution of the radiopaque medium Septal venules and filling of the capillary network zone Note the collection of the contrast medium near the tertiary airway (arrow)
Microangiograph occluded lung injection through the aorta

FIGURE 14

Left lung of a young chicken with left pulmonary artery occluded one week earlier the scale is 100 /um Late stage of contrast filling of the veins Note the millipede-like configuration (arrow) of the pulmonary venule The asterisk indicates an enlarged bronchial arteriole
Microangiograph occluded lung injection through the aorta

FIGURE 15

Left lung after the 12 days post-occlusion period in a young chicken Verhoff s elastic staining the scale is 50 μm The connective tissue in the septum is increased Two tertiary bronchioli are visible One blood vessel in the capillary network zone is indicated by an arrow Light microscopy occluded lung

FIGURE 16

The left lung three months after occlusion of the pulmonary artery in a young animal (Verhoeff s staining the scale is 50 μm) Air capillaries of various sizes are seen Small vessels are seen scattered among the air capillaries but distinctly apart from them Light microscopy occluded lung

FIGURE 17

Electron micrograph of the left lung four months after occlusion of the left pulmonary artery (scale 0 5 /um) Note the tortuous basal membrane and the long distance from the air capillaries to the blood capillaries The osmiophilic lining of the air capillary is visible (arrows)
Electron micrograph occluded lung

FIGURE 18

The left lung of a young chicken four months after occlusion of the left pulmonary artery (scale 0 5 /um) The arrow shows one laminar osmiophilic inclusion Electron micrograph occluded lung

AC

BC

ACTA PHYSIOLOGICA SCANDINAVICA
Supplementum 423

OSMOLAR CONTROL OF THE CIRCULATION IN HEMORRHAGIC HYPOTENSION

An experimental study in the cat

By
Johannes Jarhult

Lund 1975

ACTA PHYSIOLOGICA SCANDINAVICA

Supplementum 423

From the Department of Physiology and Biophysics

University of Lund

Sweden

OSMOLAR CONTROL OF THE CIRCULATION IN HEMORRHAGIC HYPOTENSION

An experimental study in the cat

By

JOHANNES JARHULT

Lund 1975

CONTENTS

CHAPTER 1 Introduction 7

CHAPTER 2 Methodology 9

CHAPTER 3 Change of plasma osmolality during hemorrhagic hypo tension 16

CHAPTER 4 Relation between hyperosmolality and hyperglycemia in hemorrhagic hypotension 22

CHAPTER 5 Roles of the sympatho-adrenal system in hemorrhagic hyperglycemia 29

CHAPTER 6 Osmotic fluid transfer from tissues to blood during hemorrhagic hypotension 37

CHAPTER 7: Autoregulation of capillary hydrostatic pressure du ring regional arterial hypo and hypertension 54

CHAPTER 8 Effects on regional vascular resistances evoked by increased blood osmolality 61

CHAPTER 9 General conclusions 66

Acknowledgements 70

References 71

This thesis is based mainly on the following publications

I JÄRHULT J J LUNDVALL S MELLANDER and S TIBBLIN Osmolar control of plasma volume during hemorrhagic hypotension Acta physiol scand 1972 85 142 144

II JÄRHULT J Osmotic fluid transfer from tissue to blood during hemorrhagic hypotension Acta physiol scand 1973 89 213 226

III JÄRHULT J and P -O GRANDE Transcapillary fluid movements in sympathectomized intestine and skin during hemorrhagic hypotension Acta physiol scand 1975 In press

IV JÄRHULT J Role of the sympatho adrenal system in hemorrhagic hyperglycemia Acta physiol scand 1975 93 25-33

V JÄRHULT J and S MELLANDER Autoregulation of capillary hydrostatic pressure in skeletal muscle during regional arterial hypo- and hypertension Acta physiol scand 1974 91 32-41

VI JÄRHULT J J HILLMAN and S MELLANDER Circulatory effects evoked by physiological increases of arterial osmolality Acta physiol scand 1975 93 129-134

In the text these papers are referred to by their Roman numerals

Chapter 1

INTRODUCTION

Hemorrhage implies a stress situation for the organism which is met by compensatory mechanisms that often are capable of restoring cardiovascular homeostasis If the compensation is insufficient and in particular if secondary circulatory derangements or other complications occur hemorrhagic shock may finally ensue Various aspects of the latter topic have been reviewed previously for instance by Wiggers (1950) Sealey and Weistger (1961) Bock (1962) Hershey (1964) Fine (1965) Mills and Moyer (1965) and Weil and Shubin (1967)

The present study deals with compensatory adjustments occurring in early stages of hemorrhage with particular reference to the development of plasma hyperosmolality and its role in cardiovascular control

The defence mechanisms in bleeding are primarily aimed at limiting the deleterious effects of hypovolemia on tissue perfusion and nutrition The rapid augmentation of sympathetic discharge leading to improved cardiac performance increased peripheral resistance and constriction of the capacitance vessels constitutes an important first line of defence but can cause no definite restoration The adequate compensation of course implies a replacement of the shed blood volume The restitution of the red cell volume is however a slow physiological process and therefore the refill of the circulatory system in the early stages of hemorrhage must be accomplished by an increased plasma volume

Previous studies have revealed different mechanisms for such restoration of plasma volume in hypovolemia Most important perhaps is a transcapillary absorption of interstitial fluid mediated by an adrenergic reflex resetting of the pre-/postcapillary resistance ratio which lowers hydrostatic capillary pressure in muscle and skin tissues (Öberg 1964) More gradual

adjustments are accomplished by the endocrine and reflex regulation of renal water and salt excretion (cf Wiggers 1950 Laragh and Sealey 1973) and by control of the fluid intake via the thirst mechanism (cf Fitzsimons 1972)

The present investigation in cats indicated that in hemorrhage there is also an important osmolar control of plasma volume related to substances other than the plasma proteins In fact this regulatory mechanism seems primarily aimed at an overall restoration of extracellular fluid volume effected by osmotic fluid withdrawal from the intracellular space A similar control principle was recently shown to be operating in heavy exercise (Lundvall Mellander Westling and White 1972)

It will be demonstrated that hemorrhagic hypotension is associated with a pronounced increase of blood osmolality (Chapter 3) in turn mainly caused by glucose release from the liver (Chapter 4) The reflex control mechanisms for this glucose liberation are described in Chapter 5 That the blood borne hyperosmolality leads to a transcapillary osmotic absorption of extravascular fluid into the blood stream is shown in Chapter 6 and this process occurs mainly in skeletal muscle and skin tissues From a quantitative point of view the osmolar plasma volume control seems to be as important as the previously known adrenergic reflex mechanism at least in severe hemorrhage Plasma volume expansion by fluid absorption does not result from the arterial hypotension per se since as described in Chapter 7 the expected passive fall of capillary hydrostatic pressure is effectively prevented by local vascula autoregulatory mechanisms Finally it is shown that the hemorrhagic hyperosmolality exerts some inhibitory action on the tone of the resistance vessels in several tissues and concomitantly a moderate positive inotropic action on the heart (Chapter 8)

Chapter 2

METHODOLOGY

Mat ri l nd ane thesia

Experiment were performed on tot l of 143 cats of both sexes (mea weight 3 2 kg) an thetl ed intravenously with α hl r lose (50 mg/kg b.wt) ft r induction with ther The maj rity of the cat also received an ini tial small do e of pentobarbit l sodium (5 20 mg) In some pilot experi me ts the an sthe I was upplemented with u than (100 mg/kg b wt) The animals we kept on normal mi ed diet and f sted fo about 16 hr before the xpe iments but rec i ed water ad libitum

S gical proced s

V scular reactions were studied in fou diff ent tissue i skelet l muscle ki int stine nd kidney

Skel tal mus l p epa tion St di s were performed on the lower leg muscles (below called calf muscl s) in 81 cats and the egion we prepared according t the techniqu de ribed by KJ llme (1964) In brief the ski of the ight hind leg we di sected f ee from the underlying muscl ti sues down t the ankl and the paw emoved t the ankle joi t The thigh muscles we sepa ted from the lowe leg The bone marrow ca ity f the dist l femu was drilled open and pl gged with cotton soaked i silicone grease to occlud intra-osseou ascul connections Small ve sels between the thigh and lowe leg we e ligated so that the popliteal rtery and vei formed the sole scul conne tion with the mai pa t of the body With few excep- tion (II VI) the muscl egion was acutely ympathectomi ed by cutti g the sci tic nerv The popliteal vein wa cannul ted and the outflow dive ted to the ext rnal jug l i i n optical drop ecorde unit When chang f ti ue volume we e to be reco ded the mu le p pa tion wa enclo ed in a wat r filled tempe t re controlled (38°C) plethysmograph si g the ki fl p for pro imal l re

I some expe iments (I II V) the t l l l flow to the calf muscles was div ted from the proximal femo l t ry ia T tube hunt to the popliteal art ry to permit local i jection of drug and precise measure- ments of a t i l l flow pre s re I 9 of these experiment (II) a pe f ion pump (Harv rd model 1210) was i se ted i the hunt to llow d

justments of the egional blood flow to desired levels In the hypotension experiments described in pape V the egional arterial inflow pressure was manually adjusted by a screw clamp proximal to the T tube

Ski preparation The hind paw was chosen fo studies of the circulatory events in a cutaneous vascular bed (20 cats) To observe reactions mainly in n triti e skin vessels the pads (with their abundant arterio-venous anastomoses) were excluded from the circulation by tight ligatures The skin of the calf was dissected free from the knee down to the ankle joint All superficial and deep vei s were ligated 1 2 cm bo e the joint except for the great saphenous vei This vessel was cannulated and its outflow di erted to the jugular vein ia a optical drop ecorder In some experi ments (VI) d ainage through deep veins was prevented by an ankle cuff the pressure in which was aised slightly above venous outflow pressure When desired the paw was sympathectomized by severing the sciatic and the femo ral nerves To ecord changes i tiss e volume (III) the paw was placed in a perspex plethysmog aph filled with water using the calf skin flap for proximal closure The water temperature was kept at 34°C (neutral tempera t re) In two experiments (III) the circulatory events in the paw were studied d ring regional hypote sion For this purpose a short shunt cir cuit was i serted between the femo al and popliteal artery all other arte- rial e sels between the thigh and the calf being ligated Regional arteri al pressure was then monito ed f om a T tube in the shunt and could be ad justed to desi ed levels by a sc ew clamp

I testinal p epa ation In 16 cats without signs of intestinal infection a p epa ation of the small intesti e was made the p inciple of which has been desc ibed by Folkow et al (1963) In brief a segment of the jejunum (weighi g 15 30 g) was isolated and the emai der of the intestines removed With some exc ption (VI) the egional a tonomic ne s we e ligated and c t The nous outflow from the segment nd its lymph node was measured with an optical drop eco de and et rned to the aninal via the external jug la vei To ecord hanges i tissue volume (III) the intestinal pre pa ation wa enclo ed i a pe spex tempe at e cont olled (38°C) pleth ysmog aph fill d with Ty ode sol tion A sho t te ial shunt ci cuit was insert d i the s pe i me enteri a te y in these plethysmographic expe iments Clo e of the pl thysmog aph at the site of the ent ance of the two cognate ssel was accompli hed with ilicone g ease

Kid y Blood flow f om the left kid ey wa eco ded in 8 expe iments (VI) Afte ligation of the l ft spermati o o a ian ei the mai renal ein was cannulat d and the enous outflow di t d i optical d op e-

corder to the ext rnal jugula v in The region l sympatheti n rves wer ligated and cut in 3 of these experiments

I t rf renc with the sympatho-ad enal ystem Observations of v sc lar ea tions we e made in the different regions both with intact sympathetic innervation and after regional sympathectomy In the latter experiments phenoxybenzamine (1 4 mg) was given close rt rially to p event an influ ence from ci cul ting catecholami es Significa t sy temic effect of the blocking drug was avoided by collecting nd discarding the venous outflow for about 3 min fter the injection This volume of blood was s bstituted by the same amount of isotoni dextran Tyrode solution The effecti eness of the α blockade was checked by close rterial i jection of norad ena line (5 10 µg/kg b wt)

I paper IV speci l attention we paid to the i fluence of different links in the sympatho-ad enal system on the glucose release from the li r during hemor hage This analy is was performed on 33 cats divided i to 4 diff rent groups In 14 cats the ympatho-ad enal system we l ft intact In 7 cats the ad enal gland wer carefully removed; in 6 cats the hepatic nerv plexus was dissected free ound the hepatic artery and portal ein ligated and cut i 6 cat the maj r nd minor planchni nerv s were ev red bilat rally and i addition both drenals emoved

Cathete ization techniques for blood sampling Befor cathet riz tion the a imals we heparinized (750 1000 IU/kg b wt) I most of the pre ent expe iments blood sampl were taken for det rminations f plasma osmol lity and pl sma glucose concent tion A t ri l sampl we e withd awn from cathet r in the right ca tid t ry and venous sampl s from the outflow cathet s i the fou diffe ent region (ee bov) In paper IV an na ly i was made of the rol of the liv in the hemo hagic hyperglycemi e ponse by det rmi ing glucose osmolality and gl cagon lev l in pl sma from ious ite in the venou ystem For thi purpose two fine ili conized polyethylen cathet we i serted i to the inf rior caval v i i the two major saphenous vein One f thes wa ad anced until it tip we positioned ju t bove the iliac bif cation (below called iliac ein) and the ther on t e ait j t bove the entrance f the hepati vei (below called hepatic ei) In some of the experiment (n=7) fi e ca thete was lso i s ted i to the port l vei via cannul tion of a di tal branch of the supe io mesent i vei

Cardiov scul r ecordi g

Mean (and sometime pulsatil) rt rial blood pressure wa mea red with

Statham P23 AC transducers placed at heart level The pressure was moni tored from the ight carotid artery or (in paper III) from the left femoral artery Regional enous outflow pressures were determined from the height of the outflow tubing above heart level or sometimes (V) measured direct ly with a Statham P23 DC tran ducer from a T tube placed close to the cann ula in the popliteal vein The transducer was then positioned at the water level in the plethysmograph to compensate f r the induced external water pressure In some of the hypotension experiments (V) the pressures in a small muscle art ry (SAP) and a small muscle vein (SVP) were monitored from drawn-out flexible nylon tubings inserted in the sural artery and ein and recorded with Statham transducers (for details see Haddy et al 1954 Lund vall 1972)

Heart rate was recorded with a G ass tachograph triggered by the sys tolic pressure wa e (VI)

The regional blood flows were continuously registered with silicone filled optical drop reco der units I serted on the venous side (Lindgren 1958)

Changes of the tissue volume we e ecorded with the plethysmographic technique originally desc ibed by Mellander (1960) The plethysmog aph de sign was modified to fit the arious tissues In some early expe iments (I II) changes of tissue volume were egistered with a piston recorde and late (III V) with n electronic g a imetric t sducer (G ass FT 10; for details see G ände et al 1974)

The ca diovascula pa ameters we e us ally ecorded on a G as polygraph or occasionally on a kymog aph

An lysis of pe ipheral cul function

Regional asc la istance was d l ed from the a te io-venous p essur g adient and egional blood flow and exp essed as mm Hg/(ml/mi 100 g tissue)

Data on capa it nce responses and et t anscapillary fluid movements we de i d f om the obse ed cha ges of tiss e olume acco ding to the principle de c ibed by Mellande (1960) This implies that abrupt changes of tiss e volume coordinated i time with chang s of asc l r sistance a e conside ed to flect the esponses of the capacitanc essels whe eas g adual changes of ti sue volume occu ing in f ce of a mai tai ed steady state esistance esponse a e conside d to ep se t net t anscapillary fl id mo ements Thi t p et tion of the volume curv has been alidated

in eve l studies by the simultaneou use of radioactive tracer techniques (e g Ablad and Mellander 1963 KJ llmer 1965)

Reaction of the prec pill ry sphincters were stimated with the capillary filtration coeffi ient (CFC) method (Cobbold et l 1963 Folkow and Mellande 1970) The venous pr s ure i crea e applied during d terminations f the CFC was less than 5 mm Hg in these experiments to mi imiz myogenic reaction (cf Mellander t al 1964) Unles otherwise stated a atio of pre-/po tcapill y istance of 4/1 wa u ed fo the CFC calculations (P ppenheimer nd Soto-Rive 1948)

Soft ti s weight of the tudied egion was det rmined at the end of the expe iment

Spread f dat is given s the tand rd ror of the mean Significance tests were performed according to Student s t test

Biochemical alyses

Pl sma osmol lity was det rmined with therml tor ryoscopy (Osmometer 31 LAS Ad anced In t uments Inc) Each sampl wa measured twi e and the mean lue sed Repetiti e eadi g on osmol r t nd rd deviated t mo t by 1 5 mOsm/kg H_2O f om the tru lue

Pl sma glucos concent tion wa det rmined by the gl cose-oxid s technique

Plasma glucagon concent ation (immuno eactive glucagon) wa estimated with a radio immuno assay technique developed by Nil son nd W llen ten (1975)

Plasma sodium and pot ssium concent tion we d t rmined with flame photometry

Pl sma urea concentration wa d termined with the Technicon Autoanalyz Sy tem

Expe imental procedure

Hemo hagic hypoten ion expe iments The animal re ted for about 30 mi ft r the compl tion of the surgical p epa tion At the end of this control pe iod t l l d venou blood samples we withd awn fo biochemical det rminations In the pl thysmogr phic t dies (I III V) venou outflow pre su in the different region wa set so s to t blish pp oxi mat ly an isovolumetri tat in the control ituation (Starli g equilib ium)

Hemo rhagic hypoten ion wa chieved by bleeding the a imal i to III

conized p essure bottle connected via a T tube to the right carotid artery The exsanguination was rapid so that arterial blood pressure reached a level of 50 mm Hg within 3 min This pressure level was then maintained throughout the exp riment with the aid of the pressure bottle Changes of the peripheral ascular functions in the different regions were followed during the hypotensive period which in most cases lasted for 90 min Repe titive arterial and venous blood samples were taken for biochemical ana lyses during the period of hypotension The animals breathed spontaneously throughout the experiments

Some comments will be gi en here concerning the abovementioned experi mental hemorrhage model This model was originally developed in Wiggers laboratory (Werle et al 1942) and has l ter been extensi ely used It implies that the animal is kept at a constant hypotensive level irrespective of compensatory adj stments The model has several advantages For instance it permits detailed studies of net transcapillary fluid movements without interference from a fluctuating arterial inflow p essure and venous outflow press re which aided in the interpretation of the present results The analysis of capillary fluid t ansf r d ring bleeding was furthermore facil itated in the present expe iments by blockade of the lymph drainage from the studied regions It should be made clear however that this bleeding model creates an artificial hemorrhagic sit ation i sofar that the animal cannot benefit from the effects of the compensatory mechanisms that nor mally tend to resto e blood pressure and volume Animals with vivid sympatho-adrenal eflexes will therefo e be exposed to a more intense vaso-const iction and hence to greate blood loss tha those with less ef fecti reflex mechanisms (cf Chien 1967) This was noticed in the present e periments i terms of a elati ely wide ange of the volume of shed blood Thus the f action of total blood volume (assumed to be 7 per cent of the body weight i the control situation see Altman and Dittmar 1971) which had to be withd awn initially to lowe blood pressu e to 50 mm Hg aried in the diffe ent a imals f om 11 35 per cent (mean alue 26 2 %)

Ca dio ascular and biochemical analyses were also pe formed in a few ham ope ted animals p epa ed as desc ibed above for the skeletal muscle region These animals we e not bled after the i itial control pe iod but left undist rbed fo 90 min

Regional hypoten ion pe iment The t ansition from normo to hypotension in the hemo hag e pe iments causes a ma ked change of the perfusion gradient and the t ansmu al p ess re i the peripher l ascula beds To analyse possibl local ffects of such p essu e alterations per se re

gional hypotension experiments we e performed (III V) I the following way The bovementioned va cul r functions were observed in e ponse to egional art rial hypotension of rying duration accomplished by dj stments of a screw clamp placed on the cogn te artery to the egion (skeletal muscle and paw) V scular esistance and CFC were determined in the isovolumet ic con trol pe iod of normoten ion and the ffects on vascula resistanc capaci tance CFC and net transcapillary fluid tran fer wer f llowed d ring periods of graded regional arteri l hypotension (ang down to 30 mm Hg) In on s ries of thes experiments rt ial p essu e i the muscl region was kept at 50 mm Hg fo period of 90 min and terial and regional venous plasma osmol lity was d termined t interv ls In some othe experiments papaverine (0 6 1 6 mg/min) we i fused cl se arterially to abolish v scu l r tone and hence active local espons s to the regional hypoten ion

An analysi of local vascular effects i skel tal muscl was also pe formed during sho t term pe iods of arte ial hype tension up to 170 mm Hg The hype ten ion was evoked by bil teral carotid occl ion after cervical agotomy Alpha ad en rgi ffects i the tudied muscle egion were then prevented by egional sympathectomy and by i se rte ial dmi i tration of phenoxybenzamine (10 mg/kg muscle tissue)

Hypertoni i f ion expe iments As will be shown hemo rhage leads to an increas f the pl sma osmolality I an ttempt to study s lectiv ly the ffects of plasma hyperosmol lity pe se on ca diov scular f nction hypertonic solutions were inf ed i t avenou ly to esting non bled imals at a r t whi h inc ea ed the rte i l osmol lity t about the same levels s i the p esent hemo hag experiment Hypertonic (30-50 %) sol tions f glucose cr se o ylose we then lowly i f sed i the right axill ary ein t rat of 0 3 1 0 ml/min In on ie of these expe iments (VI) the ffect on scula resistanc in muscl kin i t stine and kid ey and on mean and pul til arte i l pre ure and heart rate wer observed The hypertonic inf ion l ted fo 5 min nd were epeated at i tervals 2 3 time i each imal The cardiov scular parameter and arteri al pl sma osmol lity wer analy ed befo e d ing and fo 15 mi fte the hypertonic i f sion i nothe se ies of hypertoni i fu ion expe iments (Chapt r 6 and pape III) the ffect on net t anscapillary fl id movement in the sympathectomized keletal muscl paw nd int sti we observed Hypertoni glucos (30 %) we lowly i fused for 10 30 ml i these expe i ment and art ial and regional venou osmol lity determi ed at int rv ls The adrenals were emoved in these experiment to exclud ffect of po si bl releas of cathecolamin

Chapter 3

CHANGE OF PLASMA OSMOLALITY DURING HEMORRHAGIC HYPOTENSION

(Paper I IV)

General considerations

Little attention has been paid to the problem of osmolar changes in hemorr hage In 1963 Brooks et al reported that the arterial plasma osmolality in dogs was virtually unchanged after 90 min of hemorrhagic hypotension at a level of 40 mm Hg They found however an increase of the plasma glucose concentration and explained the plasma isotonicity by an equi-osmolar drop of the plasma sodium concentration Bergentz and Brief (1965) on the other hand demonstrated that severe hemorrhage in dogs in fact could be associated with a considerable increase of the arterial plasma osmolality in their experiments amounting to about 25 mOsm/kg H_2O after 20 min of ar terial hypotension at 20 mm Hg This finding in dogs was confirmed some years later by Baue et al (1967) Boyd and Mansberger (1968) and Grega et al (1971) although in the latter study the hyperosmolality was more moderate Boyd and Mansberger revealed a hyperosmolar state also in humans in hemorrhagic shock

The patho-physiological significance of the hemorrhagic hyperosmolality is poorly understood Boyd and Mansberger (1968) considered the hyperosmo-lality in dogs and patients to be a bad prognostic sign insofar that they found an impaired survival in individuals with marked plasma hypertonicity in hemorrhagic shock On the other hand Brooks et al (1963) Bergentz and Brief (1965) and Baue et al (1967) found that the survival rate in dogs was improved if the animals received a hypertonic instead of an isotonic infusion in the oligemic phase of hemorrhagic hypotension The mechanism for uch a beneficial effect however could not be revealed in these stu dies

The hypothesis is advanced that the organism might benefit from the plasma hyperosmolality during hemorrhage insofar that it might cause an osmotic fluid absorption from the extravascular to the intravascular space with a consequent increase of plasma volume Such a mechanism has recently been shown to be operating in heavy exercise (Lundvall et al 1972) An osmotic withdrawal of tissue fluid in bleeding would be possible however only if the plasma osmolality exceeds the tissue osmolality i e if the hyperosmolality is primarily blood borne As a first approach to this problem detailed studies of arterial and venous plasma osmolality were performed during hemorrhagic hypotension

Present results

Concomitant determinations of arterial and venous plasma osmolality during hemorrhagic hypotension at 50 mm Hg were made in 73 cats with intact sympatho-adrenal system (I IV and unpublished experiments) Arterial samples were always taken from the carotid artery whereas the venous samples represented different tissues viz skeletal muscle and skin intestine kidney and liver

Fig. 1 shows collected data from 63 cats on the osmolar changes in a skeletal muscle-skin region The venous samples in this series were taken from different sites popliteal vein (n=36) femoral vein (n=9) great saphenous vein (n=8) and iliac vein (n=10) The osmolality changes at these various sites in the venous system were not significantly different from each other and the data have therefore been pooled (Fig 1) In the control period, arterial plasma osmolality averaged 319 ± 0 7 and venous plasma osmolality 321 ± 0 7 mOsm/kg H_2O (in agreement with Lundvall 1972) It can be seen from Fig 1 that hemorrhage caused a marked increase of the arterial plasma osmolality with the most rapid change in the initial 10 min of hypotension. A clearcut increase could be detected as early as 45 sec after the start of

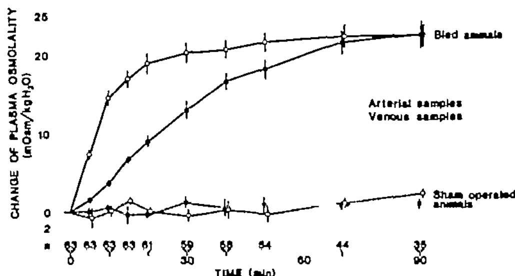


FIG 1 Changes from co t ol alue of arterial and popliteal enous plasma osmolality (mean values +S E) in cats bled to 50 mm Hg (n=63) and in sham ope ated cats (=4)

the bleeding The arterial hyperosmolality reached a value of about 20 mOsm/kg H_2O above control after 20 min a level then maintained throughout the 90 min period of observation Venous plasma osmolality on the other hand rose more gradually and did not reach the arterial level until some 60 min after the start of the hemorrhagic hypotension A positive arterio venous osmolar difference (implying a transcapillary osmolar gradient see below) was thus present during the first hour though most pronounced during the initial 30 min of hypotension The sham operated animals did not show any significant alteration of arterial or venous osmolality In some experi ments (1) the changes of arterial and venous plasma osmolality were obser ved during a more prolonged (3 hr) period of hemorrhagic hypotension at 50 mm Hg These experiments showed that the hyperosmolality remained approxi mately constant at the high level throughout this period During the last hour venous osmolality tended to slightly exceed arterial osmolality

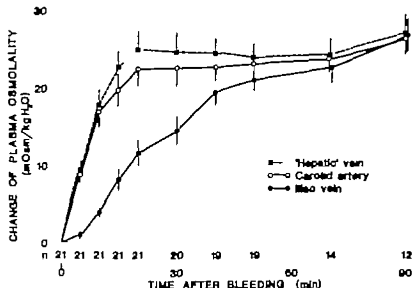


Fig 2: Simultaneously determined plasma smolality I the hepatic enou rte ial and iliac venous blood du ing hemo hagic hypotension t 50 mm Hg for 90 min Mean alues ±S E given

Data for the intestinal (n=8) and renal (n=2) circulations showed that the osmolality pattern was basically similar to that described in Fig 1 insofar that the arterial osmolality was higher than the regional venous osmolality in the initial period of hypotension In these tissues however venous osmolality approached arterial osmolality much earlier than in skeletal muscle and skin viz after 30 min in the intestine and within less than 10 min in the kidney The difference can be ascribed to the much higher blood flow in intestine and kidney than in muscle

The osmolar changes in venous samples from the hepatic vein (see Methodology) were distinctly different from those taken from the tissues mentioned From the very beginning of the bleeding the hepatic venous osmolality thus exceeded the concomitantly determined arterial osmolality and was much higher than that in the venous blood from the hindquarters (Fig 2) On the other hand the degree of hyperosmolality in the portal vein (n=7)

was much smaller than in the hepatic vein

It was concluded that the hemorrhagic hyperosmolality to a significant extent was caused by release of some substance(s) from the liver It should be stressed however that the hepatic venous blood was sampled in the inferior caval vein at the entrance of the hepatic vein thus implying considerable admixture of blood from regions other than the liver The magnitude of the true hepatic venous hyperosmolality therefore must have been underestimated in Fig 2

The data presented above refer to averages Mention should be made that 6 animals out of the 73 did not respond with significant blood hyperosmolality during the hemorrhagic hypotension

Comments

The present study has defined in detail the magnitude and the time course of the osmolar changes occurring in the arterial blood and in the venous effluent from several different tissues during hemorrhagic hypotension at 50 mm Hg In the majority of the cats (92 %) hemorrhage evoked a rapid pronounced (20 mOsm/kg H_2O) and maintained increase of arterial osmolality a finding in accordance with some previous observations in other species including man (Bergentz and Brief 1965 Baue et al 1967 Boyd and Mansberger 1968 Almli 1970 Boyd et al 1970) Our analysis indicated in *addition that the hemorrhagic hyperosmolality to a major extent was caused* by release of some osmotically active product(s) from the liver The nature of this substance will be described in Chapter 4

This investigation was made under a standardized type of hemorrhage Preliminary experiments indicated that the magnitude of the hyperosmolar response is related to the severity of the bleeding which seems to explain some discrepancies reported in the previous literature (see above)

The patho-physiological significance of the hemorrhagic blood hyperosmo

lality may be severalfold As previously reported (Almli 1970) it might affect the fluid balance in the body via an influence on the thirst mechanism It also seems to affect the internal fluid balance by causing a redistribution of fluid from the intracellular to the interstitial and intravascular compartments (Chapter 6) Hemorrhagic hyperosmolality might also influence cardiac function and peripheral vascular resistance (Chapter 8)

Chapter 4

RELATION BETWEEN HYPEROSMOLALITY AND HYPERGLYCEMIA IN HEMORRHAGIC HYPOTENSION

(Paper II IV)

General considerations

In 1877 Claude Bernard discovered that the glucose concentration in the blood rose in dogs subjected to hemorrhage a finding later repeatedly confirmed in several species (see below) This suggested to us that glucose might be responsible to a significant extent for the hemorrhagic hyperosmolality described in the previous chapter and this hypothesis was tested in the present series of experiments

Present results

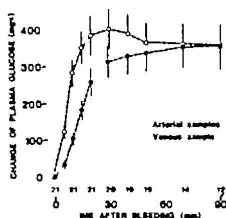
From a few pilot experiments presented in Table I it became evident that the hemorrhagic plasma hyperosmolality indeed was mainly caused by an increased glucose concentration Thus it can be seen that the gradual rise of plasma osmolality which after 70 min of hypotension exceeded the control value by 31 mOsm/kg H_2O almost entirely could be attributed to the concomitant hyperglycemia The potassium and urea concentrations also rose but the osmolar effect of these changes was more than counterbalanced by the decrease in sodium concentration Calculated values for the total osmolar change induced by these agents (neglecting the osmotic activity coefficients) are given at bottom of the table These values agreed relatively well with the observed osmolar increase except at 70 min when the observed osmolar change somewhat exceeded the calculated value

A more thorough investigation of the relation between the hyperosmolality and the hyperglycemia responses was made in 21 cats during hemorrhagic

TABLE I Changes of osmol lity glucose sodium potassium and rea-N in a teri l plasma during hemo hagic hypotension at 50 mm Hg (mean val s from 2 cats) The pproximat total change of osmol lity due to glucose sodium potassium and urea-N is depicted at bottom

	Control	Time of hypoten ion t 50 mm Hg		
		10 min	30 min	70 min
Osmol lity ($mOsm/kg\ H_2O$)	325 0	337 0 +12 0	344 5 +19 5	356 0 +31 0
Glucose (mM/L)	15 0	26 6 11 6	38 9 +23 9	41 1 +26 1
Sodium (mEq/L)	152 0	147 0 -5 0	145 5 -6 5	144 5 7 5
Pota ium (Eq/L)	3 5	6 7 +3 2	6 0 +2 5	8 5 +5 0
Urea N (mM/L)	3 3	3 6 +0 3	3 9 +0 6	4 1 0 8
Calculated smolality hange ($mOsm/kg\ H_2O$)		+10 1	20 5	24 4

hypotension at 50 mm Hg of 90 min duration (Fig 3) In the control period before bleeding arterial plasma glucose concentration averaged 220 ± 15 mg% and venous plasma glucose concentration 210 ± 14 mg% in blood drained from the hindquarters i e mainly representing skeletal muscle and skin tissues It can be seen from Fig 3 A that the arterial glucose concent ration rose quickly and reached a level of 400 mg% above control after 30 min of hypotension After this there was a slight gradual decline to about 350 mg% above control 90 min after the start of the bleeding The venous plasma glucose concentration increased more slowly and did not reach the arterial level until about 1 hour after the commoncement of the hypotension The clearcut difference between the arterial and venous plasma glucose con



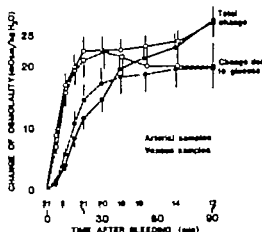


FIG 3 A Changes from control value of arterial and popliteal venous plasma glucose concentration in cats bled to 50 mm Hg (n 21) B Changes above control alue of total arterial and venous plasma osmolality in cats bled to 50 mm Hg (n 21) shown together with deduced data for osmolality changes ca sed by concomitant alteration of plasma glucose concentration Mean values ±S E gl en

centrations in the early stage of hemorrhage indicates that glucose entered the extravascular space in this period (cf Fig 3 paper II)

Fig 3 B shows the extent to which the hyperglycemia contributed to the concomitantly observed hyperosmolality in this material For this deduction 18 mg% of glucose was considered to correspond to 1 mOsm/kg H_2O It can be seen that a close correlation indeed existed between the observed changes of osmolality and the values deduced from the changed glucose concentration both in arterial and venous plasma During the initial 30 min of hemorrhagic hypotension the hyperglycemia in fact seemed to almost fully account for the plasma hyperosmolality In later stages of bleeding however the observed values for the total osmolality change clearly exceeded those calculated merely from the glucose change indicating that some other substance(s) contributed to the hyperosmolality at that time

It should be emphasized here that all data on the glucose content refer to the concentration in plasma (mg/100 ml plasma) and not in whole blood the latter unit often being used in clinical work In the control period before bleeding the plasma glucose level was about 215 mg% in these experiments which corresponds to a blood glucose level of 130 mg% taking the appropriate hematocrit value into account (and confirmed by direct determinations of the blood glucose level)

Comments

It is a well documented fact that hemorrhage is associated with hyperglycemia This has been established in several species including man (for key references see Table II) It may be noted from the data in the table that the magnitude of the hyperglycemia response seems related to the severity of the hemorrhage a phenomenon which has been analysed in some detail previously (e g Robertson 1935) Further the rate of bleeding appears to influence the magnitude of the hemorrhagic hyperglycemia (Mylon et al 1944 Carey et al 1972)

In the present investigation the hemorrhagic hyperglycemia response was confirmed The study showed in addition that the increases of plasma glucose concentration were of such magnitudes as to almost fully account for the coocomitantly observed plasma hyperosmolality especially in early stages of hemorrhagic hypotension (Fig 3 B) This finding by no means excludes the possibility that there are marked changes also of other plasma constituents during hemorrhage Thus previous studies and the present observations have shown that the concentrations of plasma potassium and various nitrogen compounds increase during hemorrhage (cf D Silva 1934 Root et al 1947 Engel 1952 and Table I) The hyperkalemia apparently is due to a loss of intracellular potassium (cf Hagberg et al 1967) and may be an effect of acidosis and/or of cell injury caused by impaired circulation

TABLE II Maximal increase of blood glucose concentration above the control level in different species during various types of hemorrhage

Species	Type of hemorrhage (BV = total blood volume before hemorrhage)	Maximal glucose increase (mg%)[x]	Time after bleeding (min)	Investigator(s)
DOG	Bled 33 % of BV	90 (V)	15	Saito et al 1928
	Bled 70 % of BV	410 (V)	150	Mylon et al 1944
	Bled 75 % of BV	255 (A)	60	Beatty 1945
	Hypotension at 60 mm Hg	480 (A)[xx]	90	Brooks et al 1963
	Hypotension at 50 mm Hg	150 (V)	180	Vigas et al 1972
	Hypotension at 40 mm Hg	460 (A)	180	Chien et al 1973
	Hypotension at 30 mm Hg	405 (A)	30	Baue et al 1967
	Hypotension at 30 mm Hg	205 (V)	90	Bauer et al 1969
CAT	Bled 18 % of BV	60 (A)	15	Brooks 1935
	Bled 32 % of BV	440 (A)	20	Robertson 1935
	Hypotension at 50 mm Hg	*400 (A)[xx]*	*30*	*Järhult 1973*
RABBIT	Bled 20 % of BV	70 (A)	10	Schenk 1894
	Bled 20 % of BV	70 (V)	125	Epstein and Baehr 1914
	Bled 33 % of BV	230 (A)	30	Ande sson 1908
RAT	Bled 50 % of BV	75 (?)	300	Engel et al 1943
SHEEP	Hypot sion at 40 mm Hg	125 (A)	75	Halmagyi et al 1966
PIG	Hypotension at 60 mm Hg	180 (V)	60	Carey and Wallack 1970
MONKEY	Hypotension at 50 mm Hg	90 (V)	120	Hiebe t et al 1973
BABOON	Bled 45 % of BV	180 (V)[xx]	60	Cerchio et al 1971
	Hypotension at 60 mm Hg	100 (V)	30	Moss et al 1970
MAN	Bled 15 % of BV	20 (V)	15	Skillman et al 1971
	(0 mm Hg at admission)	275 (V)	65	Carey et al 1970

[x] A a terial a ples V enous samples

[xx] Pl sma gl co e conce t ation

Much of the potassium seems to emanate from the liver an effect which has been attributed to released adrenaline (Shoemaker et al 1961 Andersen and Shoemaker 1967 Shoemaker 1968) The observed decrease of the plasma sodium concentration in hemorrhage is an accordance with previous findings (Brooks et al 1963 Baue et al 1967) and seems mainly to be the result of an osmotic fluid (water) withdrawal from the intracellular space (see Chapter 6) The chloride bicarbonate and hydrogen ion concentrations can change significantly in hemorrhage and minor alterations of other constituents might also occur (e g Wiggers 1950 Engel 1952 Migone 1962)

All these ionic changes in early hemorrhage seem however to virtually cancel each other with regard to the effect on total plasma osmolality In the present experiments the hyperglycemia was thus with few exceptions, found to be quite a reliable index of the increase of plasma osmolality

In later stages (> 60 min) of hemorrhagic hypotension there was a slow gradual decline of the glucose concentration in face of a maintained hyperosmolality (Fig 3 B) This decline might be interpreted as an exhaustion of the liver glycogen stores in fact some authors have reported that animals in hemorrhagic shock may die in a hypoglycemic state (e g Engel et al 1943 Strawitz and Hift 1960 Levenson et al 1961 Halmagyi et al 1966) An additional deleterious effect in this phase seems to be marked hyperkalemia In late stages of hemorrhage the present results indicate that osmotically active substances from tissues other than the liver may significantly contribute to the maintained hyperosmolality (cf also Boyd and Mansberger 1968) Such a substance no doubt is lactate which is known to accumulate gradually in plasma during prolonged hemorrhagic hypotension apparently due to impaired oxygen delivery to the tissues (e g Beatty 1945 Nastuk and Beatty 1949 Rosenberg and Rush 1968 Rokkanen et al 1971 Schweizer and Howland 1972 Chien et al 1973)

Besides the important hemodynamic effects of the glucose-induced hyper

osmolality per se (see Chapter 6 and 8) the increased plasma glucose concentration during early hemorrhage seems beneficial insofar that it helps to improve the nutritional situation for the tissues Such an effect would be of special importance for the brain the metabolism of which is highly dependant on an adequate glucose supply The relative need of glucose in over all cell metabolism might also increase in hemorrhage in view of the fact that the blood concentration of free fatty acids is often reduced after bleeding (e g Kovách et al 1970 Farago et al 1971 Spitzer and Spitzer 1972)

Chapter 5

ROLES OF THE SYMPATHO-ADRENAL SYSTEM IN HEMORRHAGIC HYPERGLYCEMIA

(Paper IV)

General considerations

Previous studies have demonstrated that the hemorrhagic hyperglycemia is caused by glucose release from the liver This was proposed as early as 1894 by Schenk who showed that the hyperglycemia response was abolished by exclusion of the liver from the systemic circulation Further support for this conclusion was later obtained from direct measurements of the blood glucose concentration in the hepatic vein (e g Robertson 1936 Shoemaker et al 1961 Bashour et al 1965 Shoemaker et al 1973) Analyses of the liver glycogen content before and after exsanguination have indicated that the glucose release is mainly due to an increased glycogenolysis (e g LePage 1946 Strawitz and Hift 1960)

The stimulus for hepatic glycogenolysis in hemorrhage has commonly been attributed to adrenaline a hormone known to effectively break down liver glycogen by activation of the adenyl cyclase system (see Sutherland and Rall 1960) Thus it has been shown that adrenaline secretion is markedly increased and that the plasma adrenaline level may rise up to 90 times the control value during hemorrhage (e g Bedford 1917 Saito et al 1928 Watts 1956 Manger et al 1957 Walker et al 1959 Walton et al 1959 Rosenberg et al 1961 Hall and Hodge 1971 Carey et al 1972) The concept of a dominant role of adrenaline in the hyperglycemia response has been strength ened by the finding that adrenalectomy can cause a decrease or abolition of the hemorrhagic hyperglycemia (e g Engel et al 1943 Halmagyi et al 1967 McCormick et al 1969) an effect present also after substitution of gluco corticoids (Hiebert et al 1973)

During the course of the present work we observed however that a hemorrhagic hyperglycemia was still present after bilateral removal of the ad renal glands suggesting a multifactorial control of the glucose release Indirect support for this hypothesis was also obtained from some recent studies showing an increased plasma glucose concentration during stimula tion of the splanchnic nerves in adrenalectomized animals (Edwards 1972a Edwards and Silver 1972 Bloom et al 1973b) In the series of experiments to be described an attempt was made to define possible different links of the sympatho-adrenal system involved in the hemorrhagic hyperglycemia response

Present results

The role of the liver in the hyperglycemia response during hemorrhagic hypo tension (50 mm Hg) was first to be established For this purpose determi nations of glucose were made in blood sampled simultaneously from the caro tid artery and from two sites in the caval vein i e at the entrance of the hepatic vein (hepatic vein) and at the iliac bifurcation Some samples were also taken from the portal and renal veins The analyses reveal ed that the plasma glucose concentration in the hepatic vein exceeded that in the arterial blood which in turn exceeded that in the iliac and portal veins (Paper IV see Fig 2) The glucose level was higher in samples taken from the renal than from the iliac vein but lower than the level in hepa tic venous blood The conclusion was therefore reached that the increased plasma glucose concentration in the present type of hemorrhage experiments is due to release of this substance from the liver in agreement with find ings in other hemorrhagic situations (see ref above)

An attempt was then made to investigate whether the hemorrhagic hyper glycemia could be attributed to hormone release from the adrenals to a sympathetic nervous influence on the liver and/or to a adrenergically in-

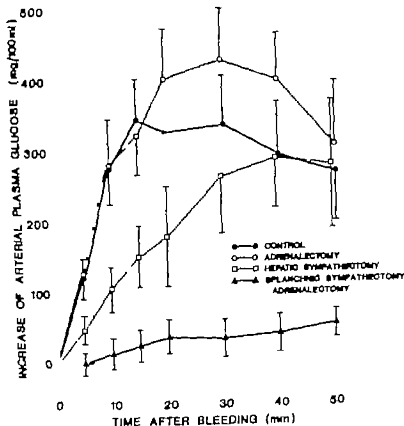
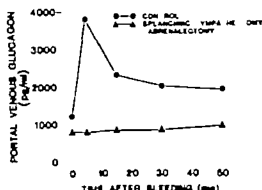


FIG 4 Inc eases of to ial pl sma glucose concentration bove the con t l level du ing hemorrhagi hypotension at 50 mm Hg I control animals (n=14) animals with adrenalectomy (n=7) animals with regional hepatic sympathectomy (n=6), and animals with pl nchnic sympathectomy combined with ad enalectomy (n=6) Mean values ±S E a e given

duced release of glucagon from the pancreas The hyperglycemia response was therefore analysed in animals with an intact sympatho-adrenal system and compared with the response after adrenalectomy after regional hepatic sympathectomy and after splanchnic sympahtectomy combined with adrenalectomy In addition the level of glucagon in the portal venous blood was determined

Fig 4 (reproduced from paper IV) shows that the arterial plasma glucose

A



B

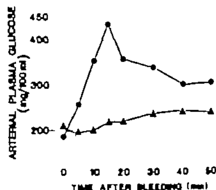


FIG 5 Mean val es for portal enous glucagon concentration (panel A) and for arterial plasma glucose concentration (panel B) during hemorrhagic hypo tension at 50 mm Hg in 2 cats with an intact sympatho adrenal system and in 2 cats with splanchnic sympathectomy + ad enalectomy

concentration rose rapidly during hemorrhagic hypotension at 50 mm Hg both in the cats with an intact sympatho adrenal system and in the adrenalecto mized animals When the hepatic nerves were sectioned the hyperglycemia response was depressed but only in the early phases of hypotension (< 30 min) However when the splanchnic nerves were cut bilaterally in adrenalectomized cats the response virtually vanished

The level of immuno reactive glucagon in portal venous plasma was determined in 2 cats with an intact sympatho-adrenal system and in 2 cats after adrenalectomy and bilateral splanchnic denervation In the intact animals hemorrhage caused an almost instantaneous rise of portal venous glucagon concentration whereas no such increase was observed after bilateral splanchnic denervation in adrenalectomized cats (Fig 5 A reproduced from paper IV) The concomitant arterial glucose concentrations are depicted in Fig 5 B showing marked differences between the two groups of animal

Comments

The present results confirm the opinion that the hemorrhagic hyperglycemia is mainly due to glucose release from the liver Taken together with previous reports they also indicate that this hepatic glucose release is mediated by at least three different mechanisms a via a direct sympathetic nerve influence on the liver b via an adrenergic release of glucagon from the pancreas; and c via a release of catecholamines from the adrenal glands The study further suggests that these mechanisms quite effectively can compensate for each other thus after removal of one link a marked hemorrhagic hyperglycemia response was still present The hyperglycemia as will be discussed below cannot be attributed to a decreased insulin secretion

Although as mentioned above most previous investigators have suggested adrenaline to be the sole cause of the glucose release in bleeding there are some earlier studies which support the proposed multifactorial hypothesis Thus Mylon et al (1944) reported that the hyperglycemia evoked by infusion of large doses of adrenaline into non bled animals was "mild compared to that seen in hemorrhage and Carey et al (1972) arrived at the same conclusion The splanchnic nerve stimulation experiments performed by Edwards group (see above) can also be taken as indirect evidence for the multifactorial hypothesis

Some details concerning the different links of the sympatho-adrenal system in the hyperglycemia response will be considered

Sympathetic nerve influence on the liver In the present hemorrhage experiments the glucose level rose by 400 mg% in adrenalectomized animals and the response was depressed after sectioning of the hepatic nerves These data suggest a direct adrenergic nervous influence on the hepatic glucose release in bleeding an opinion indirectly corroborated by the following previous observations Strong excitation of the hepatic nerves (20 Hz) in

adrenalectomized cats leads to an increase of the arterial glucose level by about 200 mg% (Edwards 1972b) Stimulation of the hepatic nerves causes an abrupt rise in the activity of several enzymes involved in liver glycogenolysis (Shimazu and Amakawa 1968a b) Further recent histochemical studies reveal a rich direct adrenergic innervation of the liver parenchymal cells at least in humans (Nobin et al unpublished)

Adrenergic glucagon release The fact that the concentration of portal venous glucagon rapidly increased after bleeding in intact animals but remained unchanged in adrenalectomized animals with severed splanchnic nerves, strongly indicates that glucagon was released via the sympatho adrenal system An analogous conclusion was made by Bloom et al (1973a) who found that glucagon could be released in the monkey in response to other types of stress Further direct excitation of the sympathetic nerves to the pancreas in cats and calves has been shown to cause a rapid glucagon liberation (Esterbuizen and Howell 1970 Bloom et al 1973b) In the latter study a close co-ordination in time was found to exist between the increase in glucagon and the subsequent increment of the arterial plasma glucose concentration The magnitude of this plasma glucose rise amounted to about 100 mg% when the sympathetic nerves were strongly activated In the present hemorrhage experiments a clearcut glucose increase occurred during the peak glucagon level but the maximum glucose rise was obtained later apparently due to additional influence from the other control systems The data taken together indicate that adrenergically induced release of glucagon is involved in the hemorrhagic hyperglycemia response although the quantitative role of this mechanism might be relatively subordinate in comparison to the others Some observations suggest that glucagon is released also in the late stages of hemorrhage but then as a result of the concomitant hypoglycemia (Halmagyi et al 1969)

Cathecholamine release from the adrenals As stated above numerous in

vestigations have demonstrated convincingly that adrenaline and noradrenaline are released from the adrenals in large amounts during hemorrhage contributing to the hyperglycemia response A detailed study of this mechanism was therefore not considered essential for the present analysis Our results of a well maintained hyperglycemia after adrenalectomy might at first sight seem to contradict this concept but in all probability can be explained by an effective compensation via the other regulatory mechanisms A significant contribution of the adrenal medullary hormones to the glucose rise in our experiments in fact seemed established by the results obtained in animals with cut hepatic nerves Under these circumstances hemorrhage evoked an arterial hyperglycemia of about 300 mg% above control but according to the findings of Bloom et al (1973b) such large a glucose increase cannot possibly be attributed to the glucagon mechanism alone

Mention should be made here that the hemorrhagic hyperglycemia (and hyperosmolality) response in animals with an intact sympatho-adrenal system was almost totally abolished after α-adrenergic blockade with phenoxybenzamine (5-10 mg/kg) but not after β adrenergic blockade with propranolol (1 mg/kg) Halmagyi et al (1968) made quite similar observations in the sheep propranolol pretreatment did not change the hemorrhagic hyperglycemia response whereas phenoxybenzamine (1 mg/kg) depressed the response These findings suggest that the liver glucose release during hemorrhage at least in the cat and the sheep mainly is mediated via α-adrenoceptors (cf Himms Hagen 1967 Hornbrook 1970)

The present study thus indicates that there are three (main) links of the sympatho-adrenal system which together contribute to the elicitation of the hemorrhagic hyperglycemia response This does not rule out the possibility that other control mechanisms can be involved as well for instance via release of growth hormone (Meyer and Knobil 1967 Carey et al 1971 Skillman et al 1971) and/or glucocorticoids (e g Sayers et al 1945

Johnson et al 1971 Cryer and Gann 1974) Since in the present experiments the hyperglycemia was virtually abolished when the splanchnic nerves were cut in adrenalectomized cats the effect of the mentioned hormones on the hyperglycemic response must have been quite small in this particular type of hemorrhage

The hyperglycemia response does however not seem to depend upon a hypothetical decrease of the insulin secretion to judge from previous studies Thus the insulin level is reported to be somewhat increased during bleeding in dogs (e g McCormick et al 1967 Vigas et al 1972) and virtually unchanged in primates including man (e g Carey et al 1970 Moss et al 1970 Cerchio et al 1971 Hiebert et al 1973) The lack of insulin release in response to hemorrhagic hyperglycemia has been attributed to an inhibitory effect of the sympatho adrenal system (e g Hiebert et al 1973 cf also Malaisse et al 1967 Porte et al 1967 Bloom et al 1973b)

It is quite likely that the hemorrhagic hyperglycemia (and hyperosmolality) response induced via the different links of the sympatho-adrenal system is reflexly controlled by cardiovascular receptors The nature of such a receptor mechanism remains to be elucidated In some preliminary experiments we observed however that the hyperglycemia and hyperosmolality responses were more closely correlated to the induced decrease of arterial blood pressure than to the magnitude of the shed blood volume This finding might be taken to indicate that the response is governed mainly by the arterial mechanoreceptors

Chapter 6

OSMOTIC FLUID TRANSFER FROM TISSUES TO BLOOD DURING HEMORRHAGIC HYPOTENSION

(Paper I III)

General considerations

It was suggested long ago that extravascular fluid enters the circulatory system after a blood loss This hypothesis was based on observations showing that the concentration of hemoglobin and/or red blood cells was lowered after hemorrhage (e g Vierordt 1854 Bierfreund 1891 Koeppe 1896) a hemodilution which seemed to occur mainly in the initial posthemorrhagic period (Boycott and Douglas 1909 Hirota 1928) The phenomenon of a partial plasma volume restoration after bleeding has been confirmed and quantitatively de fined in more detail by later studies using different techniques for plasma volume determination These investigations on animals have revealed that the main fluid replacement occurs within the first hour after bleeding (for ref see Chien 1967 see also Boyd and Mansberger 1968 Carey 1973 Chien et al 1973) and similar conclusions have been reached also from experiments in humans (e g Ebert et al 1941 Kaufmann and Möller 1958 Skillman et al 1967 a)

The mechanism for this hemodynamically important transcapillary absorption of extravascular fluid after hemorrhage was for a long time attributed solely to a passive fall of capillary hydrostatic pressure, secondary to the decli e of arterial and venous blood pressure However some indications that the sympathetic nervous system might be involved in the process were obtained by Chien (1958) He found that sympathectomy clearly diminished the hemodilution after hemorrhage and that an absorption of extravascular fluid to the blood stream occurred also after bleedings too small to cause significant changes in arterial or venous pressure

Direct experimental evidence for a net transcapillary absorption of extravascular fluid in response to activation of the sympathetic vasoconstrictor nerves was first presented by Mellander (1960) on a skeletal muscle and skin region in the cat He also showed that the phenomenon was due to a resetting of the pre-/postcapillary resistance ratio in turn causing a decrease of the capillary hydrostatic pressure Similar effects of sympathetic nerve stimulation on net transcapillary fluid exchange were observed during regional and hemorrhagic hypotension the rate of absorption however decreased with time due to impaired reactivity of the precapillary resistance vessels (Lewis and Mellander 1962 Mellander and Lewis 1963) The cardiovascular receptor control of the sympathetically mediated net capillary fluid transfer was investigated in detail by Öberg (1964) Small to moderate hemorrhage of 5-15 min duration consistently caused an augmented vasoconstrictor fibre discharge which led to mobilization of extravascular fluid to the blood stream Such an autotransfusion was present in skeletal muscle and skin but did not occur in the small intestine The effect was quantitatively most pronounced in muscle tissue and was clearly detectable even after minor blood losses Öberg also demonstrated that this reflex fluid redistribution was goverened by arterial mechano- and chemoreceptors and later studies indicate that the low pressure receptors might be involved as well (e g Öberg and White 1970) Similar studies during more prolonged periods of moderate hemorrhage showed that a fluid absorption from skeletal muscle occurred during the first 25 min and at a maximum rate of 0 1 ml/min x 100 g tissue after 5 min (Lundgren et al 1964) These results have later been confirmed in essential parts by Haddy s group (e g Haddy et al 1965 Schwinghamer et al 1970 Grega et al 1971) Reflexly induced mobilization of extravascular fluid from muscle tissue has also been demonstrated in humans after a 10 % decrease of the circulating blood volume (Mellander and Öberg 1967)

Capillary fluid transfer from the extravascular to the intravascular compartment can be accomplished by mechanisms other than those implicit in the Starling concept Hyperosmolality of non protein origin has thus been shown to cause a pronounced transcapillary fluid redistribution during heavy exercise both in animals and man (Lundvall 1972 Lundvall et al 1972) Since in early hemorrhage the tissues are perfused with blood that is hyperosmolar compared to the extravascular fluid (Chapter 3) a prerequisite exists for an analogous osmotic transcapillary fluid absorption Experiments were therefore designed to reveal such possible fluid transfer in different tissues during bleeding The analysis indicated that this mechanism indeed is present and participates in the plasma volume control during hemorrhagic hypotension

Present results

To be able to reveal possible osmotic capillary fluid transfer in hemorrhage the influences of sympatho-adrenal reflex mechanisms and of alterations of arterial and venous pressure on capillary fluid exchange must be eliminated In the experiments designed for such an analysis reflex effects were excluded by surgical and pharmacological sympathectomy and regional venous outflow pressure was kept constant The influence of the arterial pressure fall per se on the exchange process will be considered separately (Chapter 7) Observations of the capillary fluid transfer under these conditions were made during hemorrhagic hypotension in three different tissues, viz skeletal muscle skin and intestine

Skeletal muscle Fig 6 (reproduced from paper II) shows the collected data for the arterio-venous osmolar difference net transcapillary fluid movement and regional blood flow in the sympathectomized and α blocked cat skeletal muscle region during 90 min of hemorrhagic hypotension at 60 mm Hg. For comparison corresponding data from an intact muscle region are shown

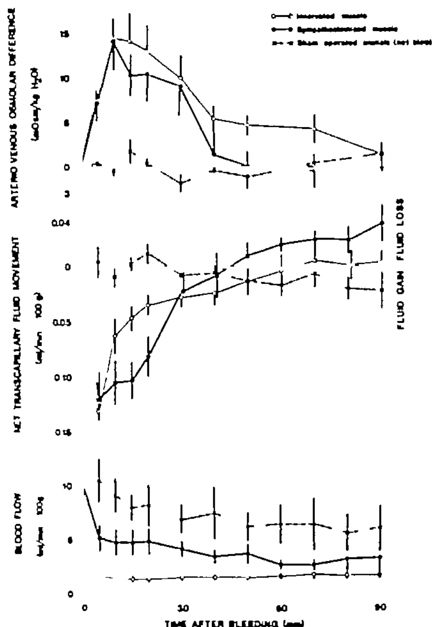


FIG 6 A te io-venous osmol difference net transcapillary fl ld movement and egional blood flow i an innervated (n 13) and a ympathectomized (14) k l t l mu cle glon of cats s bj cted to hemor hagic hypoten ion (50 mm Hg) and i sham pe ted cats (=4) Data e p essed as mean lues S E

as well as observations from 4 sham operated animals

In the sympathectomized muscle region (Fig 6 solid lines) a net transcapillary fluid absorption occurred as long as the arterio-venous osmolar difference was positive i e for about 40 min The fluid absorption was most pronounced during the initial 20 min of hypotension and the close coordination in time with the positive arterio venous osmolar difference (reflecting the transcapillary osmolar gradient) indicates that the absorption was due to osmosis (for detailed discussion see below) In the late stages of hemorrhagic hypotension (> 50 min), a moderate transcapillary fluid filtration was observed The regional blood flow (lower panel) decreased roughly in proportion to the lowered perfusion pressure if anything vascular resistance was somewhat reduced in the initial phase (during absorption) and slightly increased in the late phase of hypotension (during filtration)

The net transcapillary fluid absorption in the intact skeletal muscle region during hemorrhagic hypotension at 50 mm Hg (Fig 6 dotted lines) occurred at a somewhat slower rate in certain periods (10-20 min) than in the sympathectomized region despite the fact that the arterio-venous osmolar difference (upper panel) was quite similar in the two types of experiment This was a somewhat surprising result since it was expected that the reflex mechanism for fluid absorption operating in the intact region would add to the described osmotic fluid absorption Recent studies by Lundvall (1972) indicate however that the osmotic fluid flux in skeletal muscle is directly dependent both on the arterio venous osmolar gradient and on the magnitude of the regional blood flow Thus for a given arterio venous osmolar gradient an increase of the regional blood flow by 100 per cent was shown to lead to a corresponding increase of the osmotic fluid transfer This finding is relevant for the present analysis, since sympathectomy clearly augmented the regional blood flow (Fig 6 lower panel) Hence the data for osmotic fluid absorption (solid line) might be an overestimation of what actually occurs in the intact muscle

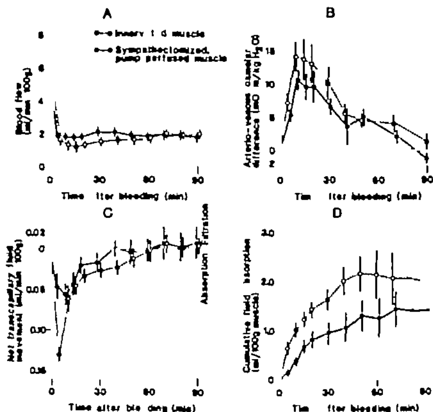


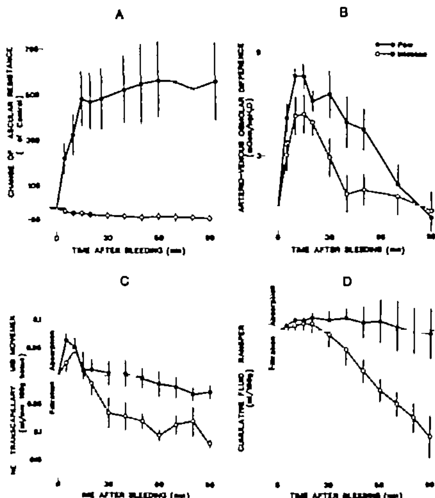
FIG 7 Comparative data in hemor hage for arterio-venous osmolar differen ce (panel B) net t anscapillary fl id movement (panel C) and cumulati e fluid absorption f om the e tra asc lar space (panel D) in the innervated (n 13) and sympathectomized (=9) muscle egion when blood flow in the latter region was mechanically adjusted to about the same level as occurred i the innervated one (panel A) Data for innervated muscle (panel A-C) sa me as i Fig 6 Mean alues ±S E gi en

This problem was approached in some experiments (Fig 7 reproduced from paper II) in which the transcapillary fluid absorption during hemorrhage was determined in the sympathectomized muscle when regional blood flow was adjusted by a perfusion pump to the same level as in the intact region (pa nel A) Since the arterio venous osmolar difference in such a pump-perfused sympathectomized region was similar to that in the intact muscle region (panel B) the observed net t anscapillary fluid transfer in the former was

considered to represent in approximate terms the true osmotic absorption of extravascular fluid in skeletal muscle during hemorrhagic hypotension of this type The net fluid absorption in the sympathectomized region was clearly diminished by the blood flow reduction (compare Fig 6 and Fig 7 C) and was now smaller than in the intact muscle (Fig 7 C) The analysis performed in Chapter 7 shows that this fluid absorption cannot be attributed to the low arterial inflow pressure per se (30-40 mm Hg) prevailing in the sympathectomized pump perfused tissue

The cumulative transcapillary fluid absorption in skeletal muscle during hemorrhagic hypotension is depicted in Fig 7 D in which the dotted curve represents total absorption and the solid curve the fraction caused by osmosis alone (derived from the absorption data in the sympathectomized pump perfused region) After 10 min of hypotension the total amount of absorbed extravascular fluid was 1 0 ml/100 g muscle (35 % osmotic) after 20 min 1 4 ml/100 g (≈ 55 % osmotic) and after 60 min 2 1 ml/100 g (60 % osmotic) It might be concluded that the adrenergic reflex and the osmotic mechanisms both are important for the plasma volume restoration after hemorrhage and that the reflex mechanism at least in the present type of bleeding is dominant in the early (< 20 min) stage of hypotension

In an attempt to further support the concept of an osmotic transcapillary fluid absorption in hemorrhage the following type of experiment was performed The osmolality of the blood was increased in non bled adrenalectomized animals (n 6) by slow intravenous infusions of hypertonic glucose solution so as to create an arterial hyperosmolality of similar nature and magnitude to that in the hemorrhage experiments The blood flow in the sympathectomized muscle region was mechanically adjusted to the same average levels as in the bleeding experiments (cf Fig 6 and 7) The rates of fluid absorption resulting from such infusions were found to agree closely with the absorption rates depicted by the solid lines in Fig 6 and 7 C espe-



IG 8 Circulatory e ents in the sympathectomized α blocked paw (●——● 8 expts) and small intesti e (o-- -o 8 expts) du i g hemor hagic hypoten sion at 50 mm Hg in the cat Panel A shows the change of ascular resistan ce pa l B the a te io-venous osmola difference panel C the net tra s capillary fl id movement and panel D the cumulati e capillary fluid trans fer Mean lues S E are given

cially in the early stage of bleeding

Skin The changes of plasma osmolality in arterial and cutaneous venous blood revealed the existance of a positive transcapillary osmolar gradient in the sympathectomized and α blocked paw during the first hour of hemorr hagic hypotension at ,0 mm Hg (Fig 8 B reproduced from paper III) The

gradient was somewhat lower than in the skeletal muscle due to a less pronounced arterial hyperosmolality in these particular experiments Observations of net transcapillary fluid movement showed that a moderate absorption of extravascular fluid occurred in the initial period of hypotension (Fig 8 C) when the arterio-venous osmolar difference was most pronounced Late in hemorrhage a slight fluid filtration was present The cumulative fluid transfer in the paw during hemorrhagic hypotension is depicted in Fig 8 D After 40 min a total of 0 8 ml of extravascular fluid per 100 g tissue had been transferred to the circulatory system but this fluid volume was returned to the tissue after 60 min of hypotension due to gradually developing filtration The vascular resistance in the sympathectomized paw increased markedly during the hypotension (Fig 8 A) most likely due to passive factors as evidenced by regional hypotension experiments (see Chapter 7) It was concluded that the fluid absorption in early hemorrhage was due to osmosis an opinion supported by hypertonic infusion experiments similar to those described for skeletal muscle

Intestine Hemorrhagic hypotension at 50 mm Hg evoked only a moderate increase of the arterio-venous osmolar difference in the small intestine (Fig 8 B) compared to that in skeletal muscle this difference was mainly due to a more rapid increase of the venous osmolality in the intestine The net transcapillary absorption in the α blocked intestine was a transient phenomenon (Fig 8 C) and the most prominent observation in fact was a marked fluid filtration in late stages of hypotension This filtration apparently was due to increased capillary hydrostatic pressure related to the gradual decline of vascular resistance (Fig 8 A) As can be seen from panel D the cumulative fluid absorption from the intestine was quite small and the main effect was a net loss of plasma fluid amounting to some 6 ml/100 g tissue after 90 min From these observations the conclusion was reached that the hemorrhagic hyperosmolality caused only a slight transient

fluid absorption from the intestine

Comments

Data on net transcapillary fluid movement in skeletal muscle skin and intestine were derived from plethysmographically recorded changes of tissue volume during constant arterial inflow pressure and venous outflow pressure. Lymph drainage from the regions was effectively prevented by careful cauterization and ligation Under these conditions a change of tissue volume can represent two events viz a net transcapillary fluid movement and/or an altered regional blood volume (capacitance response) Critical analyses of volume changes recorded with this technique (Mellander 1960 Öberg 1964 1967) have shown that net transcapillary fluid movements can be readily distinguished from capacitance responses an interpretation which has been validated by concomitant use of the ^{51}Cr-method for separate determination of changes of regional blood volume (e g Ablad and Mellander 1963 Kjellmer 1965) These analyses have further shown that the capacitance responses almost invariably are closely co-ordinated in time with reactions within the resistance vessels

The main interest in the present study was focused on net transcapillary fluid absorption and such an effect was found to occur within the first 40 min of hemorrhagic hypotension The observations of fluid absorption were made after the arterial pressure had reached a steady level of 50 mm Hg (which was accomplished in less than 3 min after the start of the bleeding) and in a state of reasonably stable tone of the resistance and hence of the capacitance vessels In some individual experiments abrupt minor changes of vascular resistance did occur but then the concomitant capacitance effects were of course disregarded in the analysis of the transcapillary fluid movement It is the efore concluded that the present approach permitted quite an accurate quantitative evaluation of the transcapillary fluid

absorption in the early stage of bleeding In the later stages where fluid filtration appeared the analysis might be somewhat less accurate since in this period more gradual minor changes of vascular resistance (and perhaps therfore also of vascular capacitance) were present The in herent error seems to be small however and possibly cannot alter the main conclusions drawn

The transcapillary fluid absorption process is of hemodynamic importance for the replacement of plasma volume in hemorrhage Such fluid absorption occurs in skeletal muscle skin and intestine but is of major quantitati ve significance only in muscle due to its very large tissue mass The main emphasis was therefore placed on the events in this tissue and the mecha nisms for the fluid absorption will be considered first

The study revealed that fluid was mobilized from the extravascular space of skeletal muscle both when the sympatho-adrenal system was intact and after regional α adrenergic blockade and sympathectomy Earlier studies (see above) have clearly shown that an adrenergic resetting of the pre / postcapillary resistance ratio occurs in hemorrhage leading to decreased capillary hydrostatic pressure and hence to fluid absorption in the in tact muscle It follows that in the α blocked region some other mecha nism(s) must have been responsible for the observed fluid absorption and we propose that it is due to osmosis caused by agents other than the plasma proteins This hypothesis is based on the following findings a The hemor rhagic hypotension was associated with a pronounced increase of arterial plasma osmolality (Chapter 3) b This hyperosmolality was mainly due to release of glucose from the liver and hence it primarily developed in the blood stream (Chapter 4 and 5); c In the period of fluid absorption clearcut arterio-venous osmolar difference existed in the tissue in turn implying the presence of a concomitant transcapillary osmolar gradient d The magnitude and time course of the transcapillary fluid absorption were

re closely related to those of the arterio-venous osmolar difference e Graded arterial hyperosmolality produced by slow i v infusions of glucose in non bled animals evoked fluid absorptions in the α blocked muscle region of similar magnitudes to those observed in hemorrhage

It might be argued that the fall of arterial pressure per se during hemorrhage would lower the capillary hydrostatic pressure and cause fluid absorption This possibility was refuted by the regional hypotension experiments to be described in Chapter 7 which showed that no significant trans capillary fluid absorption was evoked when arterial inflow pressure to the muscle region was mechanically adjusted to 50 mm Hg The maintenance of capillary pressure in this situation was mainly explained by autoregulatory adjustments of vascular tone

Several studies have shown that renin angiotensin and vasopressin can be released during hemorrhage (e g Dexter et al 1943 Ginsburg and Heller 1953 Weinstein et al 1960 Skillman et al 1967b Share 1968 Rocha e Silva and Rosenberg 1969 McNeil et al 1970 Hall and Hodge 1971 Errington and Rocha e Silva 1972) Hypothetically these vasoconstrictors might have influenced vascular tone so as to cause transcapillary fluid absorption However direct studies of capillary fluid exchange in skeletal muscle during angiotensin infusion have shown that this agent does not cause any significant fluid transfer (Folkow et al 1961 Järhult 1971) and this seems to be true also with regard to vasopressin (Diana et al 1967) Further there were no signs of significant release of such vasoconstrictor agents in the present hypotension experiments since vascular resistance in the α-blocked muscle and intestine in fact was decreased during the period of fluid absorption (Fig 6 and 8)

We therefore conclude that the transcapillary absorption of extravascular fluid from muscle tissue to the blood stream during hemorrhagic hypotension at 50 mm Hg to a significant e tent is caused by an osmotic process

mainly related to the plasma hyperglycemia The experiments also indicate that the same mechanism is operating in skin and intestine although the osmotic fluid absorption in these tissues is quantitatively less pronounced than in skeletal muscle

The question then arises from which tissue compartment the absorbed extravascular fluid emanates The released glucose molecules responsible for the hemorrhagic hyperosmolality will primarily be distributed in the blood stream but secondarily enter the interstitium and increase its osmolality as well Such an interstitial hyperosmolality must lead to significant osmotic fluid (water) withdrawal from the intracellular space since the reflection coefficient of the cell membranes for glucose can be considered to be close to unity and since the glucose transport into most cells is a slow process

Experimental support for such a fluid redistribution was obtained from the observed decrease of plasma sodium concentration after bleeding (Chapter 4) indicating a dilution and a relative expansion of the entire extracellular space This opinion is also in agreement with some direct studies of the extracellular fluid volume in early hemorrhage (e g Appelgren 1972) In fact the osmolar control system in hemorrhage might therefore basically be aimed at a restoration of the extracellular fluid volume but due to the time sequence of the hyperosmolar events it will also institute a fluid redistribution within this space from the interstitial into the intravascular compartment

Such an osmotic absorption process can be expected to be more effective across capillaries with continuous than with fenestrated endothelium due to the lower solute permeability in the former type (cf Landis and Pappenheimer 1963) The experimental observations also demonstrated such a difference with a more pronounced osmotic absorption in skeletal muscle than in intestine Even in muscle capillaries however the reflection coefficient

for glucose is far below unity (perhaps in the range of 0 2-0 3 cf Perl 1971) but evidently high enough to create opportunities for glucose-osmotic net fluid movement Yet the effective driving force for osmotic fluid movement of course is much smaller than what would be predicted from van t Hoff s law A rough estimate of this driving force (mm Hg) was obtained by dividing the observed rate of osmotic fluid absorption by the capillary filtration coefficient Such calculations indicated that the mean osmotic driving force during hemorrhage never exceeded 7 mm Hg in the α blocked skeletal muscle The magnitude of the true transcapillary osmolar gradient cannot be exactly defined in these experiments but for a given average capillary flow velocity it is related to the arterio-venous osmolar difference (for details see Lundvall 1972) At the relatively low flow states prevailing in the muscle during hemorrhage the venous osmolality would be quite representative for interstitial osmolality but the mean transcapillary osmolar gradient of course is considerably smaller than the arterio-venous osmolar difference Thus if the reflection coefficient is assumed to be 0 25 and the transcapillary glucose-osmotic effective driving force is 7 mm Hg (see above) the value for the mean transcapillary osmolar gradient can be estimated to be about 2 mOsm/kg H_2O

The present study has shown that hemorrhagic hypotension at 50 mm Hg is associated with considerable transcapillary absorption of extravascular fluid to the blood stream an event most pronounced in skeletal muscle In the intact organism this fluid absorption is accomplished by two mechanisms i e by the discussed osmolar control and by a reflex adrenergic control (cf Öberg 1964) which lowers capillary hydrostatic pressure via a resetting of the pre /postcapillary resistance ratio Quantitative data for these two processes in cat muscle tissue were given in Fig 7 D

Some aspects of the hemodynamic significance of the hemorrhagic fluid absorption will be considered below and might be most readily appreciated

by making an extrapolation of the present data from the cat to man Such an extrapolation seems to be justified for the following reasons Reflex fluid absorption from muscle and skin has been demonstrated to occur in the human being after reduction of the circulating blood volume (Mellander and Öberg 1967) and at similar rates to those in the cat taking the induced hypovolemia into account Further severe hemorrhage in man is known to evoke an arterial hyperosmolality and hyperglycemia of similar magnitudes to those reported here for the cat (Boyd and Mansberger 1968 Boyd et al 1970 Carey et al 1970) From the data given in Fig 7 D it may then be estimated that hemorrhagic hypotension at a level of 50 mm Hg would cause a total reflex fluid absorption of about 250 ml and a total osmotic fluid absorption of about 400 ml from all human skeletal muscles (30 kg) within the first hour of bleeding The entire fluid absorption from the human muscles accomplished by both mechanisms would thus comprise some 650 ml within this relatively short period of time a figure compatible with data derived from determinations of the hemodilution after comparable bleeding in man (Kaufmann and Müller 1958)

These considerations indicate that transcapillary fluid absorption in skeletal muscle constitutes a hemodynamically important principle for the plasma volume restoration in hemorrhage and that the osmolar control can be at least as effective as the reflex adrenergic regulation However the reflex mechanism is involved already at minor blood losses (cf Öberg 1964) whereas the osmotic mechanism seems to be put into play first when the bleeding is more pronounced

Osmotic fluid absorption was shown to occur also in skin and intestine (Fig 8 D) but these events contribute little to the overall plasma volume regulation Thus extrapolation of the data to the whole skin and intestinal tissues in man (each about 2 kg) indicates that the total osmotic fluid withdrawal from skin would comprise only some 20 ml and from intestine some

10 ml in severe hemorrhage

The absorbed extravascular fluid is protein-poor and leads to a decrease of the plasma colloid-osmotic pressure Yet the absorbed fluid volume is retained within the circulatory system for considerable length of time as evidenced for instance by a maintained hemodilution (e g Carey 1973 Chien et al 1973) There has been no satisfactory explanation of this phenomenon but it appears that it can be understood in view of the described osmolar fluid control Thus when intracellular water is osmotically absorbed into the interstitial space a dilution of the colloids in the interstitium of all tissues ensues and hence the interstitial colloid-osmotic pressure decreases Such an effect would tend to counterbalance the influence of a decreased plasma protein concentration on the Starling transcapillary fluid equilibrium in the body The efficiency of this mechanism increases if the interstitial protein concentration is high before dilution Recent studies have indicated that interstitial colloid-osmotic pressure indeed is significant and much higher than previously assumed (for ref see Aukland 1973 and Lönsmann Poulsen 1974)

The discussed compensatory mechanisms for plasma volume restoration in the present type of bleeding seem to be operating predominantly in the first hour of hemorrhagic hypotension In later stages however a transcapillary fluid loss was observed in all three tissues (Fig 6 and 8) particularly pronounced in the intestine Such a fluid filtration has been observed in skeletal muscle and skin in some previous studies (e g Lundgren et al 1964 Hollenberg and Nickerson 1970) and is well established phenomenon in the gut during hemorrhage (e g Johnson and Selkurt 1958 Gierert and Pedersen 1967 Cook et al 1971 Haglund 1973) This fluid loss occurred when the arterio-venous osmolar gradient had vanished and at a time when the neurogenically induced fluid absorption has been reported to be insignificant (Mellander and Lewis 1963) A similar fluid loss was also observed

after prolonged periods of regional hypotension (Chapter 7) It is suggest ed that this plasma fluid escape was caused mainly by an increased capill ary hydrostatic pressure in turn due to passively raised postcapillary re sistance Increased blood viscosity and platelet adhesiveness cell aggre gation passive collapse of the veins etc seem to be factors contributing to this effect (Hardaway et al 1962 Chien 1969 Ljungqvist 1970 Baeck ström et al 1971 Eriksson and Lisander 1972 Ericson and Eriksson 1973) In the intestine an increase of capillary hydrostatic pressure is compa tible also with the observed decline of vascular resistance (Fig 8 A) Fur ther osmotically active substances (e g lactate) seem to be produced in the cells in late stages of bleeding which also might lead to a fluid withdrawal from the interstitial and secondarily from the intravascular space By extrapolation of the present data to man the total transcapill ary fluid loss can be calculated to amount about 175 ml after 90 min of hemorrhagic hypotension of which the major part (≈ 130 ml) occurred in the intestine

It is likely that during hemorrhage in vivo the transcapillary fluid ab sorption might be somewhat larger and the fluid filtration somewhat small er than in the present experiments since central venous pressure then tends to fall as a consequence of the hypovolemia (cf Schwinghamer et al 1970 Grega et al 1971) Return of extravascular fluid from skeletal musc le and skin to the blood stream via the lymph system apparently must be quite insignificant in view of the exceedingly small lymph flow that has been observed in these tissues under normal conditions at rest (cf Jacobs son and Kjellmer 1964)

Chapter 7

AUTOREGULATION OF CAPILLARY HYDROSTATIC PRESSURE DURING REGIONAL ARTERIAL HYPO- AND HYPERTENSION

(Paper III V)

General considerations

Hemorrhage is usually associated with a fall of arterial and central venous pressure For a long time the transcapillary fluid absorption in hemorrhage was believed to be caused solely by a passive drop of capillary hydrostatic pressure (p_c) secondary to these arterial and venous pressure alterations To eliminate the effect on p_c of a possible fall of central venous pressure during hemorrhage the present analysis of transcapillary fluid movements was performed under conditions of constant venous outflow pressure in the different regions Arterial pressure however was reduced from the normal level to 50 mm Hg and it might be argued that this large pressure drop per se could have contributed to the transcapillary fluid absorption described in Chapter 6 via a passive decrease of p_c However as will be seen from the present analysis such an effect is insignificant

There are some previous observations which might be interpreted to indicate an approximate maintenance of the Starling fluid equilibrium in skeletal muscle when arterial inflow pressure is decreased (Mellander 1960 Folkow and Öberg 1961 Hanson and Johnson 1962 Lewis and Mellander 1962 Öberg 1964 Thulesius and Johnson 1966 Zweifach 1971) and such a phenomenon seems to be quite well established in the intestine (Johnson and Hanson 1962 Johnson 1968 Zweifach 1971 Haglund and Lundgren 1972 Fronek and Zweifach 1974 Gore 1974) Since skeletal muscle is the main target for the plasma volume control during bleeding there was a need for a more systematic investigation of the influence of arterial alterations per se on the muscle transcapillary fluid exchange and this was done in the present

study In view of the fact that the arterial pressure in vivo varies considerably (Bevan et al 1969) the analysis was limited not only to a pressure level of 50 mm Hg but performed over the whole range from 30 to 170 mm Hg Some observations were made in the paw as well

Present results

Skeletal muscle The effects on net transcapillary fluid movement of mechanically induced decreases of arterial inflow pressure were observed in the sympathectomized skeletal muscle with the plethysmographic technique Similar studies were also made during arterial hypertension in the α-blocked region (see Methodology)

Fig 9 shows the effects of short term (5 min) regional arterial hypo- and hypertension on net transcapillary fluid movement blood flow and vascular resistance The data are plotted versus regional perfusion pressure venous outflow pressure being kept constant at about 5 mm Hg It can be seen that such short term periods of regional hypotension did not cause any significant transcapillary fluid movement except in the lowest pressure range where in fact a slight fluid filtration was present Arterial hypertension caused minute filtration Above a perfusion pressure of 70 mm Hg there was a fairly effective autoregulation of blood flow due to adjustments of regional vascular resistance

There are no reasons to believe that tissue hydrostatic pressure or the transcapillary colloid osmotic gradient were significantly altered by the adjustments of arterial inflow pressure (for discussion see paper V) From the data on fluid transfer (Fig 9) the conclusion was therefore drawn that p_c remained approximately constant over the entire perfusion pressure range It follows that the maintenance of p_c under these conditions must be attributed to adjustments of the pre-/postcapillary resistance ratio (r_a/r_v) see Fig 10 For the calculation of r_a/r_v p_c in the control period

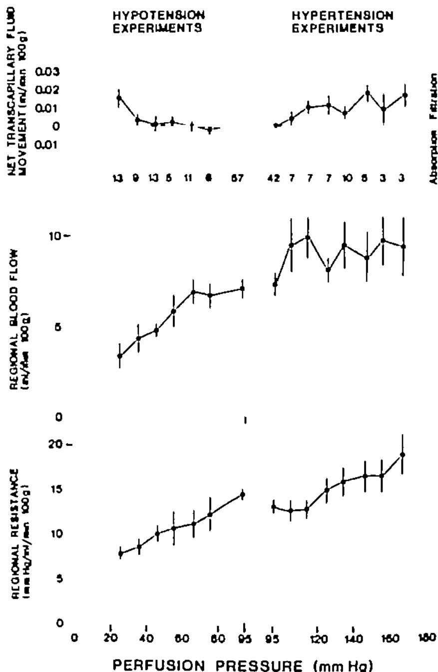


FIG 9 Cumulated mean dat ±S E on t t anscapilla y fl id movement and on esistance es el r ponses d i g short t rm glonal a terial hypo and hype te sion i cat kelet l muscle Indicat s numbe of e periment l observations

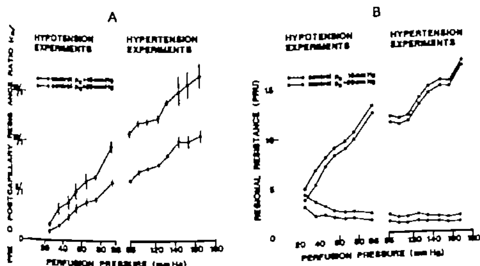


FIG 10 A. Calc lated r tios of pre /postcapill ry re istance (mean ±S E) during regional arterial hypo- and hypertension in skel tal muscl for as sumed capill ry hydrost tic pressures f 15 and 20 mm Hg pecti ly I the control pe iod with normal perf ion pre su B Ave ag precapillary (r_a) and postcapillary (r) re istan expres ed i absolute figu es (mm Hg/(ml/ in x 100 g)) du ing egional te i l hypo- and hypertension in muscle

of normotension must be known (Pappenheimer and Soto Rivera 1948) Recent studies indicate that normal P_c in the skeletal muscle at rest is in the range of 15-20 mm Hg (Diana 1970 Smaje et al 1970 Lundvall 1972 Elias sen et al 1974) and both these values were therefore used for the present calculations It can be seen (Fig 10) that r_a/r_v decreased below the con trol value during arterial hypotension and increased during hypertension (panel A) and that the alterations except in the lowest pressure range were mainly due to adjustments of precapillary resistance (panel B) The maintenance of P_c during changed arterial inflow pressure was accomplished by active autoregulatory" adjustments of vascular tone which was clearly demonstrated by comparing the results after abolition of vascular reactivi ty by papaverine infusions (paper V) In such a passive vascular bed a de-

sure but the effect was mainly caused by a decreased vascular resistance ranging from 10-30 % in the different regions (Fig 13) In relative terms the resistance decrease was most pronounced in skin and skeletal muscle

The fact that the vascular resistance decrease was present in the sympathectomized regions and also after β adrenergic blockade by propranolol (1 mg/kg b wt) indicated that the effect was caused by a direct inhibitory action of the hyperosmolality on the vascular smooth muscle (see below) The blood pressure response to hypertonicity however was depressed by about 40 % by propranolol which suggested that the effect of hyperosmolality on the heart was partly mediated via cardiac β-adrenoceptors (see further below)

Comments

The presents results showed that experimentally induced blood hyperosmolality of similar magnitude to that occurring in severe hemorrhage (Chapter 3) and in heavy exercise (Lundvall et al 1972) can evoke a moderate dilatation of peripheral resistance vessels probably by a direct action on the vascular smooth muscle The mechanisms responsible for the inhibitory action of hyperosmolality on vascular tone have been studied in vitro (Mellander et al 1967 Johansson and Jonsson 1968 Arvill et al 1969 Johansson 1969) These investigations have shown that the smooth muscle relaxation could be ascribed mainly to inhibition of myogenic pacemaker activity in turn due to changes in transmembrane ionic concentration gradients and in membrane permeabilities produced by osmotic reduction of smooth muscle cell volume It cannot be excluded however that part of the resistance decrease was due to rheological phenomena i e a lowered blood viscosity caused by absorption of extravascular fluid

The osmotically induced increase of arterial blood pressure in face of

vasodilatation in four hemodynamically important vascular beds indicates an enhancement of cardiac output in turn due to an increased stroke volume since heart rate was unchanged The increased plasma volume caused by osmotic transcapillary fluid absorption (Chapter 6) quite likely contributed to the observed blood pressure rise and then mainly via an interference with the Frank Starling mechanism The cardiac effect at least in part also might be attributed to a direct positive inotropic action of hyperosmolality a hypothesis which gains support from previous more detailed investigations both in vitro and in vivo (Koch-Weser 1963 Wildenthal et al 1969a b Templeton et al 1972 Atkins et al 1973) The attenuated blood pressure response to hyperosmolality after β blockade suggests that the cardiac effect to some extent also was mediated via β adrenoceptors an opinion corroborated by the studies of Wildenthal et al (1969a) That hyperosmolality can lead to a centrally mediated increase of sympathetic discharge has recently been shown by Hellander and Hillman (1975) and Schad and Seller (1975)

It may be concluded that hyperosmolality is a factor which can contribute to the overall cardiac adjustments in hemorrhagic hypotension but simultaneously tends to oppose the reflex peripheral vasoconstriction

The described effects were evoked by what can be considered to be a physiological increase of blood osmolality The pattern of response to hyperosmolality can be different when drastic rapid increases of blood tonicity are experimentally induced Under these circumstances a bradycardia and arterial hypotension most commonly develops (e g Binet and Stoicesco 1929 Muirhead et al 1947 Read et al 1960) but some authors have reported different neurogenic vascular resistance esponses mediated via central or peripheral receptors (Holland et al 1959 Lasser et al 1960 Agarwal et al 1969 Inglesby et al 1972 Raizner et al 1973)

Chapter 9

GENERAL CONCLUSIONS

The aim of the present investigation was to define blood osmolality changes during hemorrhagic hypotension their underlying mechanisms and their im portance for circulatory adjustments in hypovolemia The experiments were *performed on anesthetized cats exposed to standardized rapid bleeding* to a constant arterial pressure level of 50 mm Hg The following main conclu sions were drawn

1 Hemorrhage caused a rapid increase of arterial plasma osmolality reaching a level about 20 mOsm/kg H_2O above the control value after 20 min This plateau was then maintained throughout a hypotension period of 3 hours The osmolality in peripheral venous blood rose more gradually implying the existence of a positive arterio venous osmolar difference during the early stage of hypotension

2 A pronounced hyperglycemia developed during hemorrhage and its mag nitude was such as to almost fully account for the blood hyperosmolality ing the first hour of hypotension Electrolyte changes also occurred but in opposite directions (e g decreased sodium and increased potassium concentrations) so that the net effect of such alterations on total plasma osmolality was small

3 The hemorrhagic hyperglycemia was caused by glucose release from the liver in turn mediated via three different reflex mechanisms by direct sympathetic nervous influence on the liver by glucagon released via the sympatho adrenal system and by catecholamines emanating from the adrenal medulla

4 Quantitative analysis of capillary fluid exchange in muscle revealed a conside able transcapillary absorption of ext into the blood stream during the first hour of hemorrh

Such fluid transfer occurred not only in the intact muscle region but also after regional α-adrenergic blockade and sympathectomy showing that mechanisms other than the previously known adrenergic reflex resetting of the pre-/postcapillary resistance ratio contributed to the fluid absorption

5 This fluid absorption could not be attributed to a passive fall of capillary hydrostatic pressure (p_c) caused by the arterial hypotension per se, since p_c was shown to remain virtually constant at the normal control level when arterial inflow pressure to the muscle region was mechanically varied over the whole range from 30 to 170 mm Hg in non bled animals This maintenance of p_c was accomplished by local autoregulatory adjustments of precapillary vascular tone causing a resetting of the pre-/postcapillary resistance ratio

6 The fluid withdrawal was not due to a fall of regional venous pressure since this was kept constant in the present experiments Nor could it be ascribed to the effects of humoral vasoconstrictor agents such as angiotensin or vasopressin

7 The rate and time-course of the transcapillary fluid absorption in the sympathectomized and α blocked muscle region were distinctly related to the observed arterio venous osmolar difference (reflecting the transcapillary osmolar gradient) Absorption at similar rates to those observed in hemorrhage was evoked in the sympathectomized muscle region in non-bled cats in response to hypertonic glucose infusions which raised plasma osmolality to the same levels as in bleeding The conclusion was reached that the described capillary fluid transfer was an osmotic process caused by the arterial hyperosmolality (hyperglycemia)

8 Osmotic withdrawal of extravascular fluid also occurred in skin and intestine during hemorrhage This effect especially in the intestine was less pronounced than in skeletal muscle

9 The transcapillary absorption of extravascular fluid from skeletal

muscle is of great hemodynamic importance in hemorrhage insofar that it helps to restore plasma volume The present data indicated that in the intact organism the adrenergic reflex and the osmotic mechanisms are both important for this plasma volume control the former mechanism being especially effective in the initial stage of hemorrhagic hypotension Extrapolation of our data on capillary fluid transfer to man suggests that a total extravascular fluid volume of about 650 ml would be absorbed into the blood stream from all skeletal muscle tissue within the first hour of hemorrhagic hypotension The osmotic mechanism would be responsible for at least 50 % of this effect

10 It appears that the described osmolar control system is basically *aimed at an overall regulation of extracellular fluid volume in hemorrhage* insofar that it causes a glucose-osmotic fluid (water) withdrawal from the intracellular compartment The consequent dilution of proteins in the interstitial space of the tissues can help to explain why the osmotically and reflexly absorbed protein-poor extravascular fluid from skeletal muscle and skin is retained for considerable length of time within the circulatory system

11 In later stages of hemorrhage some plasma fluid was lost by filtration into skeletal muscle skin and intestine an effect most pronounced in the latter tissue

12 In addition to the effect on fluid redistribution the hemorrhagic hyperosmolality was found to exert a positive inotropic action on the heart and a moderate dilator effect on the resistance vessels in skeletal muscle skin intestine and kidney

It appears from this and previous studies that the organism with regard to its volume control has several defence mechanisms against hemorrhagic hypovolemia a Reflex adrenergic constriction of the capacitance vessels

tends to adapt almost instantaneously the size of the intravascular space in relation to the decreased blood volume and this effect is co-ordinated with reflex adjustments of cardiac performance and peripheral resistance b Reflex adrenergic resetting of the pre /postcapillary resistance ratio leads to withdrawal of interstitial fluid from muscle tissue into the blood stream hence increasing the plasma volume c The reflexly induced glucose-osmotic mechanism tends to restore extracellular fluid volume by water redistribution from the intracellular space and in addition it increases the plasma volume by net transcapillary fluid movement from skeletal muscle d By activation of the thirst mechanism and adjustments of renal excretion, the overall fluid balance in the body tends to be restored e Finally there is a gradual restitution of the red cell volume mainly effected by the erythropoietin mechanism

ACKNOWLEDGEMENTS

Many people have generously offered me help and support during the work with this thesis I wish to express my sincere gratitude

to Professor Stefan Mellander who introduced me into this field of research and whose great experience and knowledge of circulatory physiology significantly contributed to the outcome of this investigation Much of his leisure time was spent on this work

to Docent Jan Lundvall for fruitful discussions and suggestions throughout the work

to Professor Börje Johansson for valuable criticism

to Mrs Tora Zadig-Johansson Mrs Dagny Johansson Miss Berit Sjöstedt and particularly to Miss Margareta Rahm for able technical assistance and for consolation in moments of despair and standstill

to Miss Ann-Christin Isaksson for strenuous work with the typing of the manuscript without loosing her patience

to Mr Olle Hammar for skilled technical assistance

to Docent Göran Nilsson for performing the glucagon determinations

to the staff of the Department of Clinical Chemistry at the University Hospital of Lund (Head Professor Per Arne Öckerman) for kindly performing several of the biochemical analyses

This work was supported by grants from the Swedish Medical Research Council (B75-14X 2210-09A) and from the Faculty of Medicine Lund

REFERENCES

ÅBLAD B and S MELLANDER Compar tive effects of hydr lazi e sodium it rite and ac tylcholine on resistance and capacita ce blood vessels nd capillary filtr tion in skeletal muscle i the cat Act physiol ca d 1963 58 319-329

AGARWAL J B E M BAILE and W H PALMER Reflex y temic hypotension du to hypertonic sol tions in pulmonary i culation J ppl Phy iol 1969, 27 251 255

ALMLI C R Hyperosmolality accompanies hypovolemi A simple explanation of additivity of stimuli for drinking Physiol Beha 1970 5 1021 1028.

ALTMAN P L nd D S DITTMAR (ed) Respir tion and circ l tion Biological Handbook F de tions of American Soci ties f r Expe imental Biology Bethesda Maryland 1971 p 225

ANDERSEN D and W C SHOEMAKER Effect f hemorrhage on hepatic pot s ium movements Proc Soc Exp Biol Med 1967 124 840-844

ANDERSSON N Übe das Ve halten des Bl tzuckers beim Aderlas Biochem Z 1908 12 1 7

APPELGREN L Perfusion and diff sion i shock Acta phy i l scand 1972 Suppl 378 1 72

ARVILL A B JOHANSSON d O JONSSON Effects of hyperosmolarity on the volume f scul smooth muscl cell and the rel tion between cell vo lume and mus le acti ity Act phy i l scand 1969 75 484-495

ATKINS J M K. WILDENTHAL and L D HORWITZ Ca diov sc lar response to hyperosmotic man it l i anestheti ed and con cious dog Ame J Phy iol 1973 225 132 137

AUKLAND K Auto egul tion f i t r titi l fl id volume Edema-preventing mechanism Scand J Il Lab I ve t 1973 31 247 254

BAECKSTRÖM P B FOLKOW A G B KOVÁCH B LÖFVING and B ÖBERG Evidenc f pl gging of the microci cul tion f llowi g cut hemo hage 6th E rop Conf Mi rocl Aalbo g 1970 Ed by J Ditzel and D H Lewi Karg Basel 1971 pp 16 22

BASHOUR F A A Q NAFRAWI and R N McCLELLAND Spl nchni hemodynami and carbohyd t met bolism i hemo hagi shock i Shock and hypo en ion P thogen i nd treatment Ed by L C Mills and J H Moy Grune & St tton New Y rk 1965 pp 265 275

BAUE A E E T TRAGUS and W M PARKINS Effect f inc eased osmol li y and co ection f acido is on blood flow nd oxygen consumption i hemorr-

hagic shock J Surg Res 1967 7 349 356

BAUER W E S N M VIGAS R E HAIST and W R DRUCKER Insulin response du ring hypovolemic shock S rgery 1969 66 80 88

BEATTY C H The effect of hemorrhage on the lactate/pyruvate ratio and arterial-venous differences in glucose and lactate Amer J Physiol 1945 143 579-588

BEDFORD E A The epinephric content of the blood in conditions of low blood pressure and shock Amer J Physiol 1917 43 235 257

BERGENTZ S E and D K. BRIEF The effect of pH and osmolality on the production of canine hemor hagic shock S rgery 1965 58 412 419

BERNARD C Lecons sur le Diabete et la Glycogenese Animale Paris Baill e e 1877 p 210

BEVAN A T A J HONOUR and F H STOTT Direct arterial pressure recording in unrestricted man Clin Sci 1969 36 329 344

BIERFREUND M Uebe den Hämoglobingehalt bei chirurgischen Erkrankungen mit besonderer Rücksicht a f den Wiederersatz von Blutverlusten Langenbecks Arch kll chir 1891 41 1 63

BINET L and S STOICESCO Les injections intraveineuses de solutions hypertoniq es de chloru e de sodium Pa is Méd 1929 19 498 501

BLOOM S R P M DANIEL D I JOHNSTON O OGAWA and O E PRATT Release of glucagon i duced by stress Q Jl exp Physiol 1973a 58 99-108

BLOOM S R A V EDWARDS and N J A VAUGHAN The role of the sympathetic inne ation i the control of plasma gl cagon concentration in the calf J Physiol (Lond) 1973b 233 457-466

BOCK K D (ed) Shock pathogen sis d the py Springer Berlin 1962

BOYCOTT A E and C G DOUGLAS On the ca bon monoxide method of determining the total oxygen capacity and volume of blood i animals with some expe iments on anaemi and t ansf sion J Path Bact 1909 13 256 295

BOYD D R F A FOLK R E CONDON L M NYHUS and R J BAKER Predicti e alue of serum osmolality i hock following major trauma S g Forum 1970 21 32 33

BOYD D R and A R MANSBERGER J Se um wat r and osmolal changes in hemo hagi shock An expe iment i and linical study Amer Surg 1968 34 744 749

BROOKS C M The eaction of chronic spi al animals to hemorrhage Ame J Physiol 1935 114 30 39

BROOKS D K W G WILLIAMS R W MANLEY and P WHITEMAN Osmolar and elec t olyte change i haemo hagi shock L ncet 1963 1 521 527

CAREY J S Physiologi l hemodil tion i te lationships between hemodyna

ics and blood volume after acute blood loss An Surg 1973 178 87-94

CAREY L C C T CLOUTIER and B D LOWERY, Growth hormone nd drenal corti cal esponse to shock and trauma in the human Ann Surg 1971 174 451 -460

CAREY L C B D LOWEREY and C T CLOUTIER Blood sugar a d i sulin re sponse of humans in shock Ann Surg 1970 172 342 347

CAREY L C J D SAPIRA and R A CURTIN Hemor hag as timulus to adre nal epinephrin secretion B ll Soc I t Chi 1972 5 393-401

CAREY L C a d M WALLACK Blood ugar esponse to g ad d hemo hage in the pig S g Forum 1970 21 88 90

CERCHIO G M G S MOSS P A POPOVICH E BUTLER and D C SIEGEL Serum insulin and growth hormon response to hemorrhagic hock Endoc inology 1971 88 138 143

CHIEN S Quantitati ev luation of the I cul tory adj stment of splen ectomi ed dog to hemo rhage Amer J Phy iol 1958 193 605-614

CHIEN S Rol of the sympatheti rvous sy tem in hemor hag Physl l Rev 1967 47 214 288

CHIEN S Blood heology and it rel tion to flow re ist nce nd t nsca pill ry exchang with speci l f rence to shock Adv nc Mi ocl (Basel) 1969 2 89 103

CHIEN S R J DELLENBACK S USAMI D A BURTON P F GUSTAVSON and V MA GAZINOVIC Blood volume hemodynami and met boli chang s in hemo rha gi hock i normal and plenectomi ed dog Ame J Physl l 1973 225 866-879

COBBOLD A B FOLKOW I KJELLMER and S MELLANDER N rvous and local he ical control f pre-capill ry phi t re l k l t l mu l s measured by chang s i filtration coeffi ient Act phy i l scand 1963 57 180 192

COOK B H E R WILSON J and A E TAYLOR I testinal fl id l s i hem hagi hock Ame J Physl l 1971 221 1494 1498

CRYER G L and D S GANN Right t i l cept rs medi t the d enoco tical pon to hemo hage Ame J Phy iol 1974 227 325 328

DEXTER L H A FRANK F W HAYNES and M D ALTSCHULE Traumati hock VI The ff t f hemo hagi hock on the concentr tion f enl and hype ten inogen l the pl sma i unanesthetI ed dogs J Il l ve t 1943 22 847 852

DIANA J N Capill ry pre sure and filtr tion coefficient during isopro renol i f ion Ame J Physl l 1970 219 1574 1584

DIANA J N R COLANTINO and F J HADDY Tran capill ry fl id movement du ing sop l and b adyki I l f ion Ame J Physl l 1967 212

456-465

D SILVA J L The action of adrenaline on serum potassium J Physiol (Lond) 1934 82 393 398

EBERT R V E A STEAD Jr and J G GIBSON II Response of normal subjects to acute blood loss Arch I tern Med 1941 68 578 590

EDWARDS A V The sensitivity of the hepatic glycogenolytic mechanism to sti mulation of the splanchnic nerves J Physiol (Lond) 1972a 220 315-334

EDWARDS A V The hyperglycaemic response to stimulation of the hepatic sympathetic innervation in adrenalectomized cats and dogs J Physiol (Lond) 1972b 220 697 710

EDWARDS A V and M SILVER Comparison of the hyperglycaemic and glycogeno lytic r sponses to catecholamines with those to stimulation of the hepatic sympathetic i ervation in the dog J Physiol (Lond) 1972 223 571-593

ELIASSEN E B FOLKOW S HILTON B ÖBERG and B RIPPE Press re-volume characteristics of the interstitial fluid space in the skeletal muscle of the cat Acta physiol scand 1974 90 583 593

ENGEL F L The sig ificance of the metabolic changes during shock Ann N Y Acad Sci 1952 55 381 393

ENGEL F L M G WINTON and C N H LONG Biochemical studies on shock I The metabolism of amino acids and carbohydrate during hemorrhagic shock in the rat J e p Med 1943 77 397-410

EPSTEIN A A and G BAEHR Ce tai new principles concerni g the mechanism of hyperglycaemia and glycos ia J biol Chem 1914 18 21 27

ERICSON L E and E ERIKSSON Mo phological aspects of intra and extra as c l phenomena i cat skel tal muscle at low flow states Ad anc Micro- ci c (Bas l) 1973 5 62 71

ERIKSSON E and B LISANDER Low flow st t s i the micro essels of skele tal mu le i cat Acta phy iol and 1972 86 202 210

ERRINGTON M L and M ROCHA E SILVA Jr Vasop essin clearance and sec e- tion d ing haemo rhage i no mal dogs and i dogs with experimental dia bet insipid J Physiol (Lond) 1972 227 395 418

ESTERHUIZEN A C and S L HOWELL Ult astruct re of the A-cells of cat is lets of Lange hans follow g ympathetic stimulation of glucagon sec retion J Il Biol 1970 46 593 599

FARAGO G R A LEVENE T S LAU and W R DRUCKER Availability of lipid for ene gy metabolism d i g hypovolemia Su g Fo um 1971 22 7 9

FINE J Shock and pe iph al ci c lato y i sufficiency in Handbook of Physiology ed by W F Hamilton and P Dow The Williams & Wilkins Co Baltimo e 1965 Sect 2 vol 3 pp 2037 2070

FITZSIMONS J T Thirst Physiol Rev 1972 52 468-561
FOLKOW B B JOHANSSON and S MELLANDER The comparative effects of angio tensin and noradrenaline on consecutive vascular sections Acta physiol scand 1961 53 99 104
FOLKOW B O LUNDGREN and I WALLENTIN St di s on the l tionship bet ween flow resistanc capillary filt tion coeffi ient and egional blood volume in the intestine of the cat Acta physiol scand 1963 57 270 283
FOLKOW B and S MELLANDER Measurements of capillary filtration coeffi cient and its use in st die of th cont ol of capillary exchange I Capill ry P rmeability Alfred Benzon Sympo ium II ed by C Cron and N A Lassen Munksgaard Copenhagen 1970 pp 614-623
FOLKOW B and B ÖBERG Autoregulation and basal ton in consecuti e as cul r sections f the skeletal mus les in se pl e-tre ted cats Acta physiol scand 1961 53 105 113
FOLKOW B B ÖBERG and E H RUBINSTEIN A proposed diffe entiated neuro ffector organization i muscl r sistance ve s ls Angiologica 1964 1 197 208
FRONEK K and B W ZWEIFACH Pre and postcapill ry re i tanc in cat me- sentery Mi rov cul Res 1974 7 351 361
GAZITUA S J B SCOTT C C CHOU and F J HADDY Effect of osmolarity on canine enal va lar si tanc Ame J Phy iol 1969 217 1216 1223
GAZITUA S J B SCOTT B SWINDALL and F J HADDY Re i tan espon es to local hanges in pl sma osmol lity i three scula bed Ame J Physiol 1971 220 384 391
GINSBURG M and H HELLER Antidi reti acti ity i blood obtained from a ious parts f the cardiov cul sy tem J Endoc 1953 9 274 282
GLENERT J and S M PEDERSEN Albumi t an fe from blood to small intes tin i dog d i g exsang ination hypoten ion Act hi scand 1967 133 95 106
GORE R W P s re i cat mesent I t iol and capill ri du ing change i y temi t i i blood p sure Ci l t Re 1974 34 581 591
GRÄNDE P -O J JÄRHULT and S MELLANDER Method fo g a imetric egist tion f changes ti s volume Act phy i l cand 1974 91 211 215
GREGA G J J M SCHWINGHAMER and F J HADDY Changes i f relimb weight and segment i cul esi tances f llowing seve hemo rhage Ci cul t Re 1971 29 691 703
HADDY F J A G RICHARDS J L ALDEN and M B VISSCHER Small i and r- tery pre es i normal nd edematou ext emiti s of dog unde local

and general anesthesia Amer J Physiol 1954 176 355 366
HADDY F J J B SCOTT and J I MOLNAR Mechanism of volume replacement and ascular constriction following hemorrhage Amer J Physiol 1965 208 169 181
HAGBERG S., H HALJAMÄE and H RÖCKERT Shock reactions in skeletal muscle II I tracellular potassium of skeletal muscle before and after induced haemorrhagic shock Act chir scand 1967 133 265 268
HAGLUND U Vascular reactions in the small intestine of the cat during hemorrhage Acta physiol scand 1973 89 129 141
HAGLUND U and O LUNDGREN Reactions within consecutive vascular sections of the small intestine of the cat during prolonged hypotension Acta physiol scand 1972 84 151 163
HALL R C and R.L HODGE Changes in catecholami e and angiotensin levels in the cat and dog d ing hemorrhage Amer J Physiol 1971 221 1305 1309
HALMAGYI D F J., D J GILLETT L LAZARUS and J D YOUNG Blood glucose and se um insulin i e ersible and irreversible posthemo rhagic shock J Trauma 1966 6 623 629
HALMAGYI D F J M H IRVING and D J GILLETT Course of posthemorrhagic hypotension after celiac ganglionectomy with and without adrenal denervation Ann S g 1967 166 222 227
HALMAGYI D F J M H IRVING and D VARGA Effect of adrenergic blockade on the metabolic esponse to hemor hagic shock J appl Phy iol 1968 25 384 389
HALMAGYI D F J., I R NEERING L LAZARUS J D YOUNG and J PULLIN Plasma gl cagon in experimental posthemorrhagic shock J Trauma 1969 9 320 326
HANSON K M and P C JOHNSON Vascula esista ce and arterial press re in autoperf sed dog hind limb Ame J Physiol 1962 203 615-620
HARDAWAY R M W H BRUNE E F GEEVER J W BURNS and H P MOCK Studi s on the role of i t a ascular coag lation in irre ersible hemor hagic shock Ann Su g 1962 155 241 250
HAUGE A and G [illegible] Blood hyperosmolality and pulmonary ascular resistance i the cat Ci c lat Re 1971 28 371 376
HERSHEY S G (ed) Shock Little Brown and Company Inc Boston 1964
HIEBERT J M Z CELIK J S SOELDNER and R H EGDAHL Insuli response to hemor hagic hock i the i t t and drenalectomized primate Ame J Su g 1973 125 501 507
HIMMS HAGEN J Sympathetic g lation of met bolism Pharmac l R 1967 19 367 461
HIROTA K., On the r stit tion of the blood fl id afte haemorrhag J Blo

chem (Tokyo) 1928 9 87 102
HOLLAND R C J W SUNDSTEN and C H SAWYER Effects of intracarotid injections of hypertonic solutions on arterial pressure in the rabbit Ci cu lat Res 1959 7 712 720
HOLLENBERG N K and H NICKERSON Changes in p e- and po tcapillary resistance in pathogenesis of hemo rhagic shock Amer J Phy iol 1970 219 1483 1489
HORNBROOK K R Adrenergic receptors fo metabolic responses in the li er Fedn P oc 1970 29 1381 1385
INGLESBY T V A E RAIZNER H G HAWLEY nd N S SKINNER J Cardiov s cular reflexes induced by selectively altering pulmon ry rte ial osmola lity Amer J Phy iol 1972 222 302 307
JACOBSSON S nd I KJELLMER Accumulation of fl id in ex rcising skel tal mus le Act phy iol scand 1964 60 286 292
JÄRHULT J Compe ativ ffects f angi tensi and no adrenali e on esis tance cape it nce and precapill ry sphincter seis i c t kelet) mus le Act phy i i sca d 1971 81 315 324
JOHANSSON B Permeability cha te istics of vascula smooth mus le cells s evealed by thei osmotic espon es to non electr lytes Act physiol cand 1969 77 282 297
JOHANSSON B and O JONSSON Cell volume a a factor infl en ing lectrical and mechanical acti ity of sc l smooth muscl Act phy iol cand 1968 72 456-468
JOHNSON J A J O DAVIS P R BROWN J S BAUMBER nd R A WAID E iden for monophasic espon e in ldost rone and cortisol secr tion to hemorrhage P oc Soc Exp Biol Med 1971 137 1121 1125
JOHNSON P C Review of p evious tudies nd cu rent theorie of to egu l tion Ci 1 t Res 1964 14 and 15 Suppl 1 2-9
JOHNSON P C Auto eg l t ry esponses of cat mesenteric t iole mea red i ivo Ci 1 t Res 1968 22 199-212
JOHNSON P C nd K M HANSON Effect of a teri l pres u on teri l and enou re l tanc f i t tine J ppl Physiol 1962 17 503-508
JOHNSON P C and E E SELKURT I testinal weight changes in hemor hagic shock Amer J Phy iol 1958 193 135 143
JOHNSTON I H and A M HARPER The ffect of man itol on ereb l blood flow J Neuros rg 1973 38 461-471
KAUFMANN W and A A MULLER Expan ion des Pl smavolumens nach sche Ve mind ung de zi k ll enden Bl tmenge Z Krei lauffo h 1958 47 719-731

KJELLMER I The effects of exercise on the vascular bed of skeletal muscle Act physiol scand 1964 62 18 30

KJELLMER I On the competition between metabolic vasodilatation and neurogenic vasoconstriction in skeletal muscle Acta physiol scand 1965 63 450-459

KOCH WESER J Influence of osmolarity of perfusate on contractility of mammalian myocardium Ame J Physiol 1963 204 957-962

KOEPPE H., Bl tbefunde nach Aderlass Münch med Wschr 1895 42 904-905

KOVACH A G B S ROSELL P SANDOR E KOLTAY E KOVACH and M TOMKA Blood flow oxygen consumption and f ee fatty acid release in subcutaneous adipose tissue du ing hemorrhagic shock in control and phenoxybenzamine t eated dogs Ci c lat Res 1970 26 733 741

LANDIS E M and J R PAPPENHEIMER Exchange of substances through the capillary walls I Handbook of Physiology ed by W F Hamilton and P Dow The Williams & Wilkins Co Baltimore 1963 Sect 2 vol 2 pp 961 1034

LARAGH J H and J E SEALEY, The renin angiotensin aldosterone hormone system and egulation of sodium potassium and blood press re homeostatis In: Handbook of Physiology The Williams & Wilkins Co Baltimore 1973 Sect 8 pp 831-908

LASSER R P M R SCHOENFELD D F ALLEN and C K FRIEDBERG Reflex circulatory effects elicited by hypertonic and hypotonic solutions injected into femoral and b achial a te ies of dogs Circulat Res 1960 8 913-919

LePAGE G A., The effects of hemo rhage on tissue metabolites Ame J Physiol 1946 147 446 453

LEVENSON S M A EINHEBER a d O J MALM Nutritional and metabolic aspects of shock F dn Proc 1961 20 Suppl 9 99 140

LEWIS D H and S MELLANDER Competitive effects of sympathetic control a d tissue metaboli s on esistance and capacitance essels and capillary filt ation in sk letal muscl Acta physiol scand 1962 56 162 188

LINDGREN P An imp ov d method fo d op reco ding of a t ial and enous blood flow Act physiol cand 1958 42 5 11

LJUN QVIST U Platelet esponse to acute haemorrhage in the dog Acta chi sc nd 1970 S ppl 411 1 40

LÖNSMANN POULSEN H I t stitial fl id concent ations of albumin nd immunoglobulin G I normal men Scand J cli Lab Inv t 1974 34 119 122

LUCAS C E and R C READ Vascul hypertonicity A mechanism for asodilatation in the dumpi g synd ome Surge y 1966 60 395 401

LUN REN O J LUN WALL and S MELLANDER Rang of sympathetic discha ge

and reflex vascular adjustments in skeletal muscle d ring hemorrhagic hypotension Act physl l scand 1964 62 380 390

LUNDVALL J Tissue hyperosmolality as mediator of sodilat tion and t a sc pill ry fluid flux in exercising skeletal muscle Act physiol scand 1972 Suppl 379 1 142

LUNDVALL J nd J HOLMBERG Role of tissue hyperosmol lity in functional v sodilatation in the submandibular gland Acta phy iol scand 1974 92 165 174

LUNDVALL J S MELLANDER H WESTLING and T WHITE Fl id transfer between blood and tissues d ing exercise Act physiol cand 1972 85 258 269

MALAISSE W F MALAISSE LAGAE P H WRIGHT and J ASHMORE Effects of ad renergic and choli ergi agents pon insulin secretion i itro Endoc l nology 1967 80 975 978

MANGER W H J L BOLLMAN F T MAHER and J BERKSON Plasma concentration of pin phri and norepinephri e in hemo rhagic and anaphyl tic shock Ame J Physiol 1957 190 310 316

McCORMICK J R W M LIEN A H HERMAN and R H EGDAHL Glucose and i ll metabolism d ring hock in the dog Surg F rum 1969 20 12 14

McN ill J R R D STARK d C V GREENWAY Intestinal asoconst iction af t r hemo hage: roles f sopre in nd ngioten i Ame J Phy iol 1970 219 1342 1347

MELLANDER S Compar tive studi on the ad en rgi neuro hormonal control of resistanc and capacitanc blood essel i the cat Act phy iol cand 1960 50 Suppl 176 1-86

MELLANDER S and J HILLMAN Circ l t ry and respi t ry ffect evoked by hypertoni vent iculo lateral perfusion Act physiol cand 1975 i p es

MELLANDER S B JOHANSSON S GRAY O JONSSON J LUNDVALL and B LJUNG The effect of hyperosmol rity on i tact and isolated va cul smooth muscl Possible rol i ex i e hyperemi Angiologica 1967 4 310 322

MELLANDER S nd D H LEWIS Effect f hemo hagi hock on the ea ti ity of esl ta ce nd capa it nc essel and on capillary filt tion t an f i cat skel tal muscl Ci cul t Re 1963 13 105 118

MELLANDER S and B ÖBERG T capill ry fluid bso ption and the va cul ea tion i the human f rearm du ing eduction of the i cul ting blood volume Act phy i l scand 1967 71 37-46

MELLANDER S B ÖBERG and H ODELRAM Vascul r djustment t increased t ansmu al pr su i cat and man with peci l ef renc t elf l capill ry fl id t f r Act phy i l scand 1964 61 34-48

MEYER V and E KNOBIL Growth hormone secretion in the unanesthetized rhesus monkey in response to noxious stimuli Endocrinology 1967 80 163 171
MIGONE L., Metabolic aspects of shock In: Shock Pathogenesis and The py ed by K D Bock Springer Berlin 1962 pp 76 95
MILLS L C and J H MOYER (eds) Shock and hypotension P thoqenesi and treatment Grune & Stratton New York 1965
MOSS G S., G H CERCHIO D C SIEGEL P A POPOVICH and E BUTLER Serum insulin response in hemorrhagic shock in baboons Surgery 1970 68 34 39
MUIRHEAD E E., R W LACKEY C A BUNDE and J M HILL Transient hypotension following rapid intra enous injections of hypertonic solutions Amer J Physiol 1947 151 516 524
MYLON E C W CASHMAN Jr and M C WINTERNITZ The relation of adrenalin and of the carotid sinus to the hyperglycemia of shock Amer J Physiol 1944 142 638 647
NASTUK W L and C H BEATTY Standardization of hemorrhagic shock in the dog Ame J Physiol 1949 156 202 209
NAVAR L G A C GUYTON and J B LANGSTON Effect of alterations in plasma osmolality on renal blood flow autoregulation Amer J Physiol 1966 211 1387 1392
ÖBERG B Effects of cardiovascular reflexes on net capillary fluid trans fe Acta physiol scand 1964 62 suppl 229 1 98
ÖBERG B The elationship between acti e constriction and passi e recoil of the vel s at arious distending p essures Acta physiol sc nd 1967 71 233 247
ÖBERG B and S WHITE The ole of vagal cardiac nerves and arterial baro ecepto s i the circulatory adjustments to hemorrhage in the cat Acta physiol cand 1970 80 395-403
PAPPENHEIMER J R and A SOTO-RIVERA Effecti e osmotic pressure of the plasma proteins and other quantities associated with the capillary circu lation i the hindlimbs of cats and dogs Amer J Physiol 1948 152 471-491
PERL W Modified filt ation permeability model of tran capillary tran sport a sol tion of the Pappenheimer po e puzzle? Mic ov scul Re 1971 3 233 251
PORTE Jr D A ecepto mecha ism for the inhibition of insulin lease by pl ephri e in ma J cli I est 1967 46 86 94
RAIZNER A E J C COSTIN R P CROKE J B BISHOP T V INGLESBY and N S SKINNER Jr Reflex systemic and local hemodynamic alterations with experimental hyperosmol lity Ame J Phy iol 1973 224 1327 1333

READ R C J A JOHNSON J A VICK nd H W MEYER Vacul effects of hypertonic solutions Cl cul t R s 1960 8 538 548

ROBERTSON J D The effect of haemor hage of rying deg ee on blood and plasma volume on blood sugar nd on art ial blood-pr e J Phy l l (Lond) 1935 84 393 409

ROCHA E SILVA J M and M ROSENBERG The release f vasopre in l e-ponse to haemor hage and it role in the mechanism of blood p ssure re-gul tion J Physl l (Lond) 1969 202 535 557

ROKKANEN P M LAHDENSUU J KATAJA V MÄKELÄ and C-G GREF T aumatic and haemorrhagl hock in bbit Ann Chi Gy Fen 1971 60 224 234

ROOT W S J B ALLISON W H COLE J H HOLMES W W WALCOTT and M I GRE GERSEN Dist rbanc in the hemistry and in the acid base balance of the blood of dogs in hemor hagic and t aumatic shock Ame J Phy l l 1947 149 52 63

ROSENBERG J C R C LILLEHEI J LONGERBEAM nd B ZIMMERMANN Studies on hemo rhagl and endotoxin shock in l tion to vasomotor changes nd en dogenou l cul ting pineph l norepinephrine and erotonin An Surg 1961 154 611 628

ROSENBERG J C nd B F RUSH Blood lacti acid lev ls in l rever ibl hem-o rhagi and l thal endotoxin hock Su g Gynec Obst t 1968 126 1247 1250

SACHS R G H G HANLEY and N S SKINNER Jr K^+ smol lity and subcu taneou adipos tissu blood flow Pflüg Ar h ges Phy iol 1971 327 337 348

SAITO S B KAMEI and H TACHI Simultaneou d termination f the epineph rine liber tion and the suga content and coagul tion time f blood l the non f stened non anaestheti ed dog ft haemorrhage Tohoku J exp Med 1928 11 205 217

SAYERS G M A SAYERS T Y LIANG nd C N H LONG The chol sterol and as co bi acid content of the ad enal liver b l and pl sma following hemor hag Endoc inology 1945 37 96 110

SCHAD H nd H SELLER Influence of l t anl l osmotic timuli on enal rv acti ity l anae theti ed cat Pflüg Ar h ges Phy iol 1975 353 107 121

SCHENK F Uebe den Zuckergehalt d Bl t nach Blutentziehung Pflüge s Arch g Phy l l 1894 57 553-572

SCHWEIZER O and W S HOWLAND Hemorrhagi hock. Cli Anesth 1972 2 101 124

SCHWINGHAMER J M G J GREGA and F J HADDY Ski and muscl l cul tory

MEYER V and E KNOBIL Growth hormone secretion in the unanesthetized rhe-
s s monkey in response to noxious stimuli Endocrinology 1967 80 163 171

MIGONE L., Metabolic aspects of shock In: Shock Pathogen sis and Therapy
ed by K D Bock Springer Berlin 1962 pp 76-95

MILLS L C and J H MOYER (eds) Shock and hypotension Pathogenesis and
treatment Grune & Stratton New York 1965

MOSS G S., G M CERCHIO D C SIEGEL P A POPOVICH and E BUTLER Serum in
sulin response in hemorrhagic shock in baboons Surg ry 1970 68 34 39

MUIRHEAD E E., R W LACKEY C A BUNDE and J M HILL Transi nt hypotension
following rapid intravenous injections of hypertonic solutions Amer J
Physiol 1947 151 516-524

MYLON E C W CASHMAN Jr and M C WINTERNITZ The relation of adrenalin
and of the carotid sinus to the hyperglycemia of shock Amer J Phy-
siol 1944 142 638-647

NASTUK W L and C H BEATTY Standardization of hemorrhagic shock in the
dog Amer J Phy iol 1949 156 202 209

NAVAR L G A C GUYTON and J B LANGSTON Effect of alterations in plasma
osmolality on enal blood flow auto egulation Amer J Physiol 1966
211 1387 1392

ÖBERG B Effects of ca diovascular reflexes on net capillary fluid trans
fer Act physiol scand 1964 62 suppl 229 1-98

ÖBERG B The elationship between active const iction and passi e recoil
of the veins at va ious distending pressures Acta physiol scand 1967
71 233 247

ÖBERG B and S WHITE The role of agal cardiac ner es and arterial baro
receptors in the ci c latory adjustments to hemorrhage in the cat Acta
physiol scand 1970 80 395-403

PAPPENHEIMER J R and A SOTO RIVERA Effective osmotic pressure of the
plasma p otei s and othe quantities associated with the capillary circu
lation in the hindlimbs of cats and dogs Ame J Physi l 1948 152
471 491

PERL W Modified filt ation permeability model of tra scapillary tr
spo t a solution of the Pappenheimer pore puzzle? Mic ov scul Res
1971 3 233 251

PORTE Jr D A ecepto mechanism fo the inhibition of insulin release
by pineph i e i ma J ll i est 1967 46 86 94

RAIZNER A E J C COSTIN R P CROKE J B BISHOP T V INGLESBY and N S
SKINNER J Reflex ystemic and local hemodynamic alterations with
experimental hyperosmolality Ame J Phy iol 1973 224 1327 1333

hagic shock in the r t Surg Forum 1960 11 112 114
SUTHERLAND E W and T W RALL The relation of adenosine 3 5 phosphate and phosphoryl se to the actions of catecholamin s and othe hormones Pharmacol Rev 1960 12 265 299
TEMPLETON G H J H MITCHELL and K WILDENTHAL Influence of hyperosmola lity on l ft ventric la stiff ess Amer J Physiol 1972 222 1406-1411
THULESIUS O nd P C JOHNSON P e- and postcapill ry resistance in skeletal muscle Amer J Physiol 1966 210 869-872
VAN HEERDEN P D H N WAGNER J and S KAIHARA Intestinal blood flow during pe f sion of the j junum with hypertonic glucose in dog Ame J Phy i l 1968 215 30-33
VIERORDT K Beit äg zur Physiologie des Blutes Arch physiol Heilk 1854 13 259-287
VIGAS M R E HAIST F BAUER and W R DRUCKER Insulin secretion during hemor hagic shock in dogs Endoc exp 1972 6 231 237
WAHL M W KUSCHINSKY O BOSSE and K THURAU Dependency of pial arterial and arteriolar diameter on pe i scular osmol rity in the cat Circulat Res 1973 32 162 169
WALKER W F M S ZILELI F W REUTTER W C SHOEMAKER D FRIEND nd F D MOORE Ad enal medullary ec ti i hemor hagic hock Ame J Physiol 1959 197 773 780
WALTON R P J A RICHARDSON R P WALTON Jr and W L THOMPSON Sympathetic influence du ing hemo rhagic hypotension Amer J Physiol 1959 197 223 230
WATTS D T Arterial blood pineph ine level du ing hemo rhagic hypoten sion i dogs Ame J Phy i l 1956 184 271 274
WEIL M H and H SHUBIN (ed) Di gnosi and t eatment of hock, Williams nd Wilkins Baltimo e 1967
WEINSTEIN H R M BERNE and H SACHS V sopressin in blood; effect of hemo rhag Endoc inology 1960 66 712 718
WERLE J M R S COSBY and C J WIGGERS Observ tions on hemorrhagic hypo ten ion and hemorrhagi shock Ame J Physiol 1942 136 401-420
WIGGERS C J Physiology of hock The Commonwealth Fund New York 1950
WILDENTHAL K D S MIERZWIAK and J H MITCHELL Acute effects of inc eased serum osmol lity on l ft ent icul pe formance Ame J Physiol 1969a 216 898 904
WILDENTHAL K C L SKELTON and H N COLEMAN III Ca diac muscle mechani l hyperosmotic sol tions Ame J Physiol 1969b 217 302 306

YOUNG D B and H H ROSTORFER Blood flow and filtration rate re
alterations in renal arterial osmolarity Amer J Physiol
1003 1008

ZWEIFACH B W Local regulation of capillary pressure Circulat
28 and 29 Suppl I 129-134

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 4[illegible]4

INTERACTION BETWEEN CENTRAL NEUROGENIC MECHANISMS DESIGN

Experimental studies in spontaneously hypertensive rats

by

MARGARETA HALLBÄCK

Göteborg 1975

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 424

From the Department of Physiology
University of Göteborg, Sweden

INTERACTION BETWEEN CENTRAL NEUROGENIC MECHANISMS *AND CHANGES IN CARDIOVASCULAR DESIGN* IN PRIMARY HYPERTENSION

Experimental studies in spontaneously hypertensive rats

by

MARGARETA HALLBACK

Göteborg 1975

This summary is based on studies reported in the following papers:

I Cardiovascular responses to acute mental stress in spontaneously hypertensive rats M Hallbäck and B Folkow Acta physiol scand 1974 90 684-698

II Consequence of social isolation on blood pressure cardiovascular reactivity and design in spontaneously hypertensive rats M Hallbäck Acta physiol scand 1975 In press

III Background of increased flow resistance and vascular reactivity in spontaneously hypertensive rats B Folkow M Hallbäck Y Lundgren and L Weiss Acta physiol scand 1970 80 93 106

IV The distensibility of the resistance vessels in spontaneously hypertensive rats (SHR) as compared with normotensive control rats (NCR) M Hallbäck Y Lundgren and L Weiss Acta physiol scand 1974 90 57 68

V Consequences of myocardial structural adaptation on left ventricular compliance and the Frank-Starling relationship in spontaneously hypertensive rats M Hallbäck O Isaksson and E Noresson Acta physiol scand 1975 In press

The papers are referred to in the text by their Roman numerals

CONTENTS

INTRODUCTION 5

HEMODYNAMICS OF PRIMARY HYPERTENSION 6

Early or labile hypertension 6

Established hypertension 7

Background of reduced resistance vessel radius 8

Hemodynamic consequences of altered design of the resistance vessels; 10

THE NEUROGENIC COMPONENT IN PRIMARY HYPERTENSION 12

The defence reaction 14

METHODOLOGICAL CONSIDERATIONS 16

Experimental animals 16

Blood pressure and heart rate measurements 16

Arrangements for acute psychological stress stimulation 17

Hindquarter perfusion procedures 18

Perfusion medium; 18

Technical arrangements; 19

Isolated heart perfusion 21

Statistical evaluations 22

RESULTS AND COMMENTS 23

Cardiovascular responses to acute psychological stress 23

Structural adaptation of the resistance vessels in SHR 27

Structural adaptation of the heart in SHR 34

GENERAL DISCUSSION 37

SUMMARY AND CONCLUSIONS 44

ACKNOWLEDGEMENTS 46

REFERENCES 47

INTRODUCTION

The background of the increased arterial pressure in essential hypertension has been subjected to numerous investigations but no sole or main cause has been revealed so far (cf Pickering 1968) It rather appears as if this disease of regulation" (Page and McCubbin 1965) is the result of an altered interaction between the various mechanisms involved in normal blood pressure regulation in such a way as to reset the pressure equilibrium to a higher level in essential hypertension Although many different opinions exist concerning the etiology of essential hypertension most investigators nowadays agree on the presence of several predisposing hereditary elements (cf Miall 1971) It is here of interest that also the most common types of hereditary hypertension in rats developed by selective inbreeding (Smirk and Hall 1958 Okamoto and Aoki 1963) are characterized by a polygenic inheritance Among these animal models of man s essential hypertension the Okamoto type of spontaneously hypertensive rat (SHR) has been particularly well studied, exhibiting several close parallels to the situation in man with an important involvement of particularly neurohormonal mechanisms (cf Okamoto 1972 Folkow 1975)

For such reasons SHR were utilized in the present series of studies which are below denoted I V The main purpose was to elucidate the relative roles and temporal interactions of functional and structural elements for the initiation and maintenance of the hypertensive state Study I was designed to explore possible differences between SHR and normotensive control rats (NCR) concerning centrally induced cardiovascular responses to graded alerting stimuli and if a hyperreactivity in this respect could constitute a genetically linked factor predisposing for hypertension in SHR Study II was aimed to elucidate whether normal environmental influences per se in the animal s daily life interact with the mentioned hyperreactivity in such a way as to contribute crucially to the gradual initiation of the hypertensive state

Although the results suggest that such an interaction of central excitatory influences seems to exert a most important triggering effect it was evident from earlier studies both in man (Folkow 1956 Conway 1963 Sivertsson 1970) and in SHR (cf Weiss 1974 Lundgren 1974) that the secondary but rapid establishment of structural cardiovascular changes appears to be a prerequisite for the creation of chronic hypertension Interest was therefore also focused on the extent and type of structural adaptation of the resistance vessels (III+IV) and its importance for the maintenance of an increased flow resistance and pressure in SHR hypertension

Finally many studies in man indicate that the hemodynamic situa-

tion in primary hypertension may often start as a hyperkinetic circulatory state with an increased cardiac output (for ref see Julius and Schork 1971) to be gradually transferred into a state of normal or even slightly lowered cardiac output but with a raised resistance (e g Eich et al 1966) It was therefore explored in SHR whether this shift in hemodynamic balance along with progression of hypertension also might depend on the gradual cardiovascular structural adaptation but then rather on the development of left ventricular hypertrophy

HEMODYNAMICS OF PRIMARY HYPERTENSION

Early or labile hypertension

Although nomenclature and definitions vary considerably this phase of hypertension is generally defined as the state when arterial pressure values vary around the borderline between normal and abnormal Therefore the term borderline hypertension is frequently used (cf Julius and Shork 1971) Already in 1939 Wezler and Böger recognized in young patients with hypertension that the increased blood pressure was sometimes accounted for by an increased cardiac output (CO) later confirmed by e g *Werkö and Lagerlöf (1949) Widimský Fejfarová and Fejfar (1957)* Bello Sevy and Harakal (1965) Sannerstedt (1966) Lund Johansen (1967) Julius and Conway (1968) Frohlich et al (1970) This increased cardiac output observed in 30-50 per cent of patients with labile hypertension (for ref see Julius and Schork 1971) is sometimes ascribed to an increased stroke volume (Widimský Fejfarová and Fejfar 1957 Finkielman Worcel and Agrest 1965 Bello Sevy and Harakal 1965) but more commonly to an increased heart rate (e g Sannerstedt 1966 Lund Johansen 1967 Julius and Conway 1968 Frohlich et al 1970) or a combination of both (Eich et al 1966) However the relationship *between cardiac output and oxygen consumption seems to be largely* normal both during rest and during exercise in labile hypertension (Julius and Conway 1968 Lund Johansen 1967 Sannerstedt 1966) The regulation of cardiac output appears therefore to be essentially normal but starts from a higher baseline level (Sannerstedt Julius and Conway 1970)

The mechanisms behind the increased cardiac output or hyperkinetic circulatory state present in the resting situation are not fully understood It has been proposed that a primary volume expansion and thus increased venous return leads to an increased cardiac output and then to a secondary rise of resistance that is assumed to be due to whole body autoregulation (Coleman Granger and Guyton 1971) Such a train

of events may well occur in case a primary volume increase really is at hand However subjects with early essential hypertension appear to display a normal or even reduced circulating blood volume (e g Bello Sevy and Harakal 1965 Julius et al 1971 a) pointing to other types of initiating mechanisms In fact most subjects with labile hypertension display obvious signs of an increased sympathetic discharge to the heart in association with a decreased vagal activity (Julius Pascual and London 1971) In addition they seem to show a centralization of their reduced blood volume per se (Ellis and Julius 1973) with a consequently increased filling of the heart a phenomenon which may be indicative of an increased sympathetic activity to the cardiovascular system notably to its venous side (Walsh Hyman and Maronde 1969 Brod et al 1974)

Together these findings strongly indicate an early involvement of an increased central neurogenic drive to the cardiovascular system by its balance suggesting a mild defence reaction which seems to be present in many though by no means in all cases of early essential hypertension (cf Folkow 1975) Peripheral resistance was earlier thought to be largely normal in labile hypertensives However when related to their raised cardiac output they display elevated total peripheral resistance compared with normotensives with similar resting cardiac output (Julius and Conway 1968) An increased flow resistance in labile hypertensives was also evident during mild exercise (Sannerstedt 1966 Lund-Johansen 1967) Therefore it has been stated that a disturbed balance between cardiac output and resistance is responsible for the pressure elevation rather than the raised cardiac output alone (e g Widimský Fejfarová and Fejfar 1957 Julius et al 1971 b)

Established hypertension

Already more than two hundred years ago a well conceived concept of what is now called essential hypertension was described by Samuel Schaarschmidt (cf Backer 1953) However since no technique for indirect blood pressure measurement was available at that time investigations mainly relied upon postmortem examinations of the cardiovascular system Thus when Bright (1836) described a number of cases with degenerative kidneys later called chronic Bright s disease he noted among other things a marked left ventricular hypertrophy Later investigations (Johnson 1868 Gull and Sutton 1872 Ewald 1877) also revealed an altered morphology of the arteries and arterioles which was even considered to be the primary or essential condition of the chronic Bright s disease Mahomed (1881) being one of the first to measure arterial pressure clinically demonstrated the presence of an increased pressure in most cases of Bright s disease He also proposed that the cardiovascular changes

may precede the renal changes which was also considered by e g Huchard (1889) Thus the concept that hypertension can precede kedney lesions is much older than the term essential hypertension which was introduced first 1911 by Frank

Already Bright found an increased resistance to flow in diseased kidneys (cf Backer 1953) and since then an increased systemic resistance to flow has been considered the main feature in established essential hypertension Cardiac output is by and large normal although somewhat changed in its distribution with a modestly increased supply to the skeletal muscles at the expense of the renal and splanchnic vascular beds (cf Fries 1960 Brod et al 1962 Pickering 1968) According to Poiseuille s law resistance to flow is determined by the radius length and number of the tubes and the viscosity of the perfusate There are good reasons to assume that effective blood viscosity is largely normal in established essential hypertension (for ref see Pickering 1968 Lundgren 1974 Weiss 1974) Concerning vascular dimensions in hypertension an increased length of the resistance vessels has not been convincingly documented (cf Pickering 1968) and an average reduction of vascular diameter is by far the main determinant of flow resistance particularly since resistance varies inversely to the fourth power of the internal radius Therefore most studies of the hemodynamics in established hypertension have been concentrated on the background of the reduction in average resistance vessel radius

Background of reduced resistance vessel radius

It was earlier taken more or less for granted that the raised resistance in established essential hypertension was largely a matter of a sustained in } ease in vascular smooth muscle activity and the main controversy was how such an assumed increase of smooth muscle activity was induced Thus it has been suggested to be caused e g by increased neurogenic or hormonal excitatory influences by a raised smooth muscle sensitivity to such extrinsic influences or/and by an increased myogenic tone perhaps resulting from a changed ionic balance across the muscle cell membranes Little if any attention was paid to the possibility that the structural changes in arteries and arterioles which have been recognized for more than 100 years could be of crucial importance for the raised resistance until this aspect was taken up for critical analysis some 20 years ago (for ref see Folkow et al 1973)

Concerning the abovementioned alternatives for increasing smooth muscle activity it seems clear that mechanisms of this nature are likely to play a role as initiating factors and furthermore that they probably differ in nature in the various types of hypertension For example hormonal influences are clearly of crucial significance in some secondary types of hypertension like renal hypertension Conn s syndrome Cushing s

disease and pheochromocytoma (cf Pickering 1968)

The increased resistance may as previously mentioned also be the result of altered ionic composition of the vascular smooth muscles (cf Friedman Friedman and Nakashima 1957 Pickering 1968) or/and reflect a water logging of the vessel wall (Tobian and Binion 1952) In SHR it has been suggested that an altered ionic transport in vascular smooth muscle of large arteries may account for an increased reactivity to noradrenaline (Jones 1973) However essentially all studies of e g SHR vascular strips reveal a normal or slightly reduced reactivity to noradrenaline (see below) Moreover there is no difference in sodium potassium and water content in SHR arteries in the very early prehypertensive stage suggesting that any electrolyte disturbances which may appear later are rather a consequence than the cause of hypertension (Nagaoka Kikuchi and Aramaki 1970)

Concerning neurogenic effects which will be discussed separately below there are several findings indicating enhanced sympathetic influences both in essential hypertension in man and in SHR However it is difficult to judge whether such quantitative differences really reflect a tonic increase in sympathetic activity or rather a more translent increase as a result of a raised central reactivity in the hypertensive subjects when exposed to the apprehension and discomfort of investigation Hence the obtained differences in neurogenic influences between groups might rather reflect differences during experimental conditions that are not necessarily present during truly resting conditions (cf Pickering 1968 Folkow 1975)

A great many studies illustrate how most hypertensive subjects are hyperreactors to a variety of vasoactive substances where predominantly the adrenergic transmitter noradrenaline has been used (for ref see Doyle 1968 Mendlowitz 1973) The background of such a changed vascular reactivity can be complex indeed (cf Johansson 1974) but such findings generally have been taken to reflect either a genetically linked hypersensitivity/reactivity of the smooth muscle cells per se (e g Doyle and Frazer 1961) or an inherent abnormality of noradrenaline metabolism (e g Mendlowitz et al 1962 Mendlowitz 1973) However the increased vascular reactivity can also be due to an altered design of the resistance vessels in the direction of an increased wall/lumen ratio as will be dealt with in more detail below Thus while a vascular hyperreactivity is no doubt at hand when tested as resistance changes (cf Doyle 1968) most studies performed on vascular smooth muscle strips a technique which eliminates the impact of a changed wall/lumen ratio report a largely equal or even a reduced responsiveness to e g noradrenaline in strips from cases with primary hypertension and normotensive controls (e g Spector et al 1969 Clineschmidt et al 1970 Hallbäck Lundgren and Weiss 1971 Horwitz et al 1974) Furthermore in

quantitative studies of the threshold sensitivity of the resistance vessels to noradrenaline which are here more relevant than preparations from large arteries no signs of any altered smooth muscle sensitivity to the adrenergic transmitter have been observed either in human essential hypertension or in SHR (Sivertsson 1970, Finch and Haeusler 1974) However vascular hyperreactivity was clearly observed which suggests an altered design of the resistance vessels

Hemodynamic consequences of altered design of the resistance vessels

As mentioned media hypertrophy was recognized in hypertensive disease long before its modern classification by Johnson (1868) and Ewald (1877) who even speculated whether the structural arteriolar changes might be crucially involved in the very establishment of the hypertensive state Since then the presence of an increased wall thickness reducing the wall/lumen ratio of the arterioles in hypertension has been well documented by numerous morphological studies (e g Kernohan Anderson and Keith 1929 Moritz and Oldt 1937; see also Pickering 1968)

Thus although media hypertrophy has generally been considered to be the main cause of the increased wall/lumen ratio in hypertensive arterial vessels other types of changes have also been suggested such as an increased intramural water content i e "water logging (Tobian and Binion 1952) or a vascular contracture" (Short and Thomson 1959, Short 1966) Furthermore with morphological techniques the problems of quantitation are great and a number of artifacts has to be considered e g differences in smooth muscle activity at the moment of fixation difficulties to define comparable sections of the vascular bed differences in transmural pressure and vascular distensibility etc Such difficulties were to a great extent avoided by Short (1966) in his careful studies Suwa and his group have also very elegantly solved most of these difficulties (Furuyama 1962 Suwa and Takahashi 1971) The latter group found a close correlation between blood pressure and degree of media hypertrophy in arteries and precapillary resistance vessels with the exception of their most distal sections which were considered to be protected from any pressure rise by the resistance increase in more proximal sections

Thus it can be concluded that an increased wall thickness leading to an enhanced wall/lumen ratio in the resistance vessels in hypertension has been well documented with modern morphological techniques However such methods can hardly compete in accuracy with hemodynamic evaluations of vascular dimensions since with the latter approach the measured variations in flow are amplified to the fourth power of changes in internal vascular radius Further the transmural distending pressure can be far better defined as can also the level of smooth muscle activity Moreover with morphological studies only occasional vessels can be sampled for

statistical analysis whereas the hemodynamic approach automatically provides information about the average change in design of all the resistance vessels

However when evaluating the structurally determined component of the increased resistance in hypertension by the hemodynamic approach it is naturally all-important to first eliminate all extrinsic and intrinsic interferences with smooth muscle activity First when they are completely relaxed an adequate baseline is provided from which smooth muscle activations can be induced and their effects on dimensions distensibility etc of the resistance vessels can be estimated In other words in situations of maximal dilatation and standardized transmural pressures the flow resistance reflects the resting length of the circumferentially arranged smooth muscle coat This level forms the necessary starting point for judging the extent of smooth muscle contraction by the induced increase in resistance Thus only when comparing the resistance during maximal dilatation with that during normal basal levels of smooth muscle activity in different vascular beds it is possible to see whether this basal vascular tone differs in the various vascular beds (cf Celander and Folkow 1953 Löfving and Mellander 1956)

Concerning hypertension an increased forearm (or hand) resistance to flow has been demonstrated both during rest i e at basal vascular tone and when vasodilatation was induced by nerve blockade indirect heating or by arterial occlusion (Prinzmetal and Wilson 1936 Pickering 1936) However at that time a vascular hypertonus rather than structural changes was considered to cause the raised resistance The importance of structural vascular changes for maintaining an increased flow resistance in hypertension was brought into focus when Folkow (1956) Folkow Grimby and Thulesius (1958) and Conway (1963) utilized the principles of analysis outlined above for comparing different systemic circuits and by special procedures ensured a complete smooth muscle relaxation of the forearm resistance vessels Thus quantitative comparisons of this regional resistance at maximal dilatation and in the resting situation in patients with essential hypertension and in normotensive controls could be performed These quantitative hemodynamic measurements in the forearm in association with the morphologically well documented media hypertrophy in hypertension illustrate how an increased wall thickness when encroaching upon the vascular lumen raises the resistance even during maximal dilatation Since e g neurogenic activations of the media are initiated from its adventitial layer where the neuroeffector junctions are concentrated (cf Johansson et al 1970) the reduction of the internal radius becomes exaggerated in vessels with a thicker media because of the greater bulk of tissue that is forced towards the lumen upon smooth muscle shortening (Folkow 1956 Folkow Grimby and Thulesius 1958) These exaggerated resistance increases thus

imply a vascular hyperreactivity that does not necessitate any smooth muscle hyperreactivity or -sensitivity

To summarize in the presence of media hypertrophy an increased resistance can be maintained in hypertension at a normal activity of the smooth muscle per se because first the very baseline for their opera tion is raised and second a given muscle shortening leads to exaggerated luminal reductions thanks to the changed design In fact amplified variations in resistance both in the direction of constriction and dilata tion will result from the presence of a structural increase in wall/lumen ratio as long as no sclerotizing processes are also involved (for further details concerning theoretical considerations see Lundgren 1974 Weiss 1974 and study III)

THE NEUROGENIC COMPONENT IN PRIMARY HYPERTENSION

From what has been mentioned above it follows that the extent of resistance change in hypertension does not directly reflect the extent of vasoconstrictor fibre activity since the more the wall/lumen ratio of the resistance vessels is structurally increased the more will such a factor amplify the resistance changes Therefore when comparing the extent of adrenergic fibre activity in hypertensives and normotensives by analyzing the effector response it is necessary to use effectors where possible changes of design will not affect the response like heart rate or venous capacitance response Even then the presence of e g an ector supersensitivity may result in exaggerated response to a given of fibre discharge

However the reduced venous distensibility observed in patients with essential hypertension in all likelihood reflects an increased sympa thetic activity (Walsh Hyman and Maronde 1969 Brod et al 1974) Such an interpretation is supported by an analysis of the venous compart ment in SHR hypertension where there is no evidence of any altered design or true effector supersensitivity (cf Folkow et al 1974) Further analyses of cardiac neurogenic control in early hypertension (e g Julius Pascual and London 1971) strongly indicate the presence of an increased sympathetic activity in essential hypertension of man

Another reasonably good indicator of overall changes in sympathetic activity can be obtained by measurements of the release of the sympa thetic transmitter noradrenaline (NA) provided that the amount released per stimulus and its rate and mode of elimination has not been changed Initially the urinary NA excretion rate was measured since it was found to increase during generalized increases in sympathetic activity as in duced by tilting exercise mental stress etc (cf Euler 1956) In

many investigations normotensive and hypertensive subjects were compared in this respect but with rather inconsistent results (for ref see Brunjes 1964 De Quattro and Miura 1973) With the development of sensitive methods for measuring catecholamines in blood a more direct reflection of sympathetic activity was available and most results with this technique suggest a moderately increased sympathetic activity in patients with essential hypertension (Engelman Portnoy and Lovenberg 1968 Engelman Portnoy and Sjoerdsma 1970 Louis and Doyle 1971 Louis et al 1974) The plasma concentration of dopamine β-hydroxylase (DBH) which converts dopamine to noradrenaline in the nerve endings and is released upon sympathetic activation has also been demonstrated to be higher in hypertensives than in normotensives (Louis et al 1974) although the range of variability in resting normotensives may be too large to allow for any reliable quantitation (e g Rush et al 1974 see also De Quattro and Miura 1973)

Direct recordings of sympathetic fibre activity to the vessels of skeletal muscle and skin have recently been performed in man (Wallin Delius and Hagbarth 1973) and the general pattern of sympathetic dis charge appears to be qualitatively the same in hypertensive and normotensive subjects Although so far the technique has not allowed any clear-cut quantitative separation between the two groups the findings illustrate like those of Wagner Wackerbauer and Hilger (1968) Korner et al (1974) and others the resetting of the arterial baroreceptor reflex in human essential hypertension which was first shown experimentally in dogs and rabbits with renal hypertension (McCubbin Green and Page 1956 Aars 1969) An intact baroreceptor function but reset to a higher pressure level has also been demonstrated in SHR (Nosaka and Okamoto 1970 Nosaka and Wang 1972)

With SHR as an animal model for primary hypertension the in volvement of neurogenic mechanisms has been extensively explored using morphological histochemical as well as physiological methods In general these studies suggest an increased neuro-hormonal rather than a purely neurogenic participation involving not only the sympatho-adrenal system but also the ACTH corticoid and TSH thyroxin systems (cf Okamoto 1969 1972) Furthermore in young anesthetized SHR exposed to surgical interventions an increased heart rate and cardiac output have been demonstrated which seem in part to be due to an increased sympathetic activity to the heart (Pfeffer 1972 Pfeffer et al 1974) Very young SHR also seem to display an increased cardiac NA turnover when compared with NCR (Yamori 1974) However most comparative studies on older SHR and normotensive controls suggest if anything a lower NA turnover rate in SHR hearts (Louis et al 1969 Okamoto 1972) The advantage with this approach even though indirect and with several pitfalls is that the average degree of sympathetic activity can be

followed during conditions when the animals are largely undisturbed Other evidence indicating an increased cardiac sympathetic activity in young SHR is the effectiveness of early β-adrenergic blockade treatment preventing blood pressure to increase when administered to young pre-hypertensive animals while the same treatment is relatively inefficient in older SHR (Weiss 1974 Conway 1975)

When combined the above mentioned findings indicate an increased neurogenic influence on the cardiovascular system in both human and SHR hypertension at least in early phases It is hardly possible to reveal the exact nature of the enhanced autonomic discharge i e whether it is tonic and persistent in nature or mainly episodic and intermittent However what has so far been revealed concerning the enhanced neurohormonal activity in early SHR hypertension and in early hypertension in man with signs of e g an increased sympathetic and decreased parasympathetic activity to the heart (Julius Pascual and London 1971 Pfeffer 1972) *closely resembles the pattern normally* elicited via corticohypothalamic autonomic centres during arousal i e the defence reaction

The defence reaction

Experimental studies of mild emotional stress in man (e g forced mental arithmetics) have shown an increased cardiac output increased forearm blood flow associated with cutaneous and renal vasoconstriction resulting in an increased arterial pressure (Brod 1963) This hemodynamic pattern corresponds in virtually all details to the neurohormonal adjustments ved in animals during the defence reaction (Eliasson et al 1951 *Abrahams Hilton and Zbrożyna 1960) Like several other hypothalamic* response patterns this reaction preparing for fight or flight involves both autonomic and hormonal adjustments suited to meet the particular demands that the situation may call for and these adjustments often precede the ultimate motor response They include a central vagal inhibition to the heart in association with an increased sympathetic activity to the heart venous side and most vascular circuits except to that of the skeletal muscles where instead sympathetic cholinergic vasodilator fibres and/or adrenaline secretion contribute to a powerful vasodilatation (cf Folkow and Neil 1971) The balance between the vasodilatation in skeletal muscles and the vasoconstriction elsewhere usually results in a largely unchanged or even reduced systemic resistance and the venoconstriction increases cardiac filling (Folkow Mellander and *Öberg 1961 Cobbold et al 1964)*

Since cardiac output generally increases (Folkow et al 1968) arterial pressure is more or less raised which then activates the arterial

baroreceptors but their reflex inhibition of the heart is almost totally surpressed (Hilton 1963 Lisander 1970) The reflex modulation of resistance to flow is however hardly at all surpressed by the defence reaction particularly not in the skeletal muscles with the result that cardiac out put is enhanced and favours the skeletal muscles without too drastic pressure increases (Lisander 1970)

The hormonal adjustments in the defence reaction include release of adrenaline from the adrenal medulla (Grant et al 1958) and an intense activation of the ACTH-corticoid system (e g Folkow et al 1967) Furthermore associated with the renal vasoconstriction a neurogenic renin release appears to ensue (cf Davies 1973 Zanchetti and Stella 1975) thereby involving also the angiotensin-aldosteron mechanisms

Intermittent stimulation of the hypothalamic defence area in rats with chronically implanted electrodes leads to a gradual increase in resting blood pressure which was not observed in equally treated but unstimulated controls (Folkow and Rubinstein 1966) The importance of frequently elicited defence reactions for the gradual initiation of a raised arterial pressure level has further been demonstrated in genetically normotensive laboratory animals exposed to chronic stress (e g Henry Meehan and Stephen 1967 Herd et al 1969 Smookler et al 1973 Alexander 1974)

As mentioned above the labile phase of hypertension in man frequently exhibits a closely similar hyperkinetic circulation like that seen during mild defence reactions in animals Since the question of an enhanced adrenergic activity in essential hypertension has been much debated several investigations have been designed to evaluate whether hypertensive subjects react with more intense defence reactions than normotensives when under emotional stress Thus young patients with essential hypertension tend to increase their urinary catecholamine excretion more than normotensives during mental stress (Nestel 1969) It has also been observed that hypertensives display more pronounced tachy cardia (Kalis et al 1957) as well as more longlasting and intense blood pressure increases when compared with normotensive controls in connection with stressful conversation or forced mental arithmetics (e g Brod 1963) Similar signs of a cardiovascular hyperreactivity have also been reported in SHR exposed to prolonged stress (Yamori et al 1969)

METHODOLOGICAL CONSIDERATIONS

Experimental animals

Throughout the present experiments the spontaneously hypertensive rat (SHR) of the Okamoto strain (Okamoto 1969) was used as an experimental model for essential hypertension in man (see Introduction) Originally Okamoto and his group developed SHR by selective inbreeding of Wistar rats of the Kyoto strain choosing animals with arterial pressure levels above normal and subsequent generations were obtained through brother-sister mating A small stock of SHR was supplied from NIH to this laboratory in 1968 and breeding has been continued here although not always with brother sister mating in order to diminish the extent of disturbing passenger phenomena not relevant for the hypertensive state per se

SHR hypertension develops gradually with age and mean arterial pressure is slightly above normal already at the age of 6 weeks Thus different phases of this primary hypertension can be classified according to the age of the animals The age around 6-10 weeks corresponds to an early what may be called prehypertensive phase The animals show a gradual increase in pressure and may be classified as having a fully established hypertension at the age of 6-8 months The rate of pressure rise and mean arterial pressure levels at different ages vary somewhat with different substrains and presumably also with the methods and general conditions during which the pressure measurements are performed

In study I renal hypertensive rats (RHR) were also used Renal hypertension was induced in 6-7 week old normotensive male Wistar rats by lacing a standardized silver clip on the left renal artery during ether anesthesia leaving the right kidney intact About 70 per cent of the operated rats developed marked hypertension within 3 weeks after operation Hypertensive rats (blood pressure ≥ 150 mm Hg) were used for experiments 4 weeks after operation

Blood pressure and heart rate measurements

All pressures given throughout the present studies are mean arterial pressure as measured intra-arterially When measured in awake animals that are left undisturbed and are properly handled the intra-arterially recorded mean arterial pressure is in all likelihood the most reliable way of estimating the true resting pressure level The technique used in studies I II and V was as follows The rats were lightly anesthetized with ether while the caudal artery was cannulated In the animals used

for subsequent stress stimulation (studies I and II) Xylocain® containing 2 per cent of adrenaline was applied around the incision which was then sutured and the tail was bandaged with tape These procedures were used to avoid any irritation from pain and the animals usually appeared to be fairly undisturbed by the arrangements The rats were then allowed to wake up from the ether anesthesia and mean arterial pressure and heart rate were recorded in the freely moving animal via a long arterial cannula which was connected to a Statham pressure transducer and a Grass polygraph No difference was obtained whether mean arterial pressure was recorded 5 or 60 minutes after the animals had woken up from ether anesthesia Heart rate in SHR was however somewhat lower and more stable 60 minutes compared with 5 minutes after anesthesia while the normotensive control rats (NCR) were less affected in this respect

In study III mean arterial pressure was measured in the cannulated carotid artery under standardized Nembutal® anesthesia (3 mg/100 g body weight i p) Tracheal cannulation was then also performed to allow free airways before the pressure was recorded However as repeatedly tested the tracheal intubation and the cannulation of one carotid artery tended to increase the pressure level considerably even though most baroreceptor sites remained intact Thus when measuring the mean pressures in the cannulated tail artery in awake otherwise intact animals the subsequent exposure to Nembutal® anesthesia unilateral carotid occlusion and tracheotomy led to a pressure increase of 49±6 mm Hg in SHR and 25±7 mm Hg in NCR Therefore the definitely higher arterial pressures in both SHR and NCR in study III compared to the other studies is mainly a consequence of these procedures However this did not to any greater extent increase the ratio between the mean arterial pressures in SHR and NCR which in this particular case was the relevant point In study IV the mean arterial pressure although recorded via the tail caudal artery was measured during Nembutal® anesthesia but information concerning mean arterial pressure in the awake situation was obtained here by indirect blood pressure measurements performed prior to the actual experiment (for details see Weiss 1974)

Arrangements for acute psychological stress stimulation

In studies I and II the cardiovascular responses to different types of alerting stimuli (acute psychological stress) were evaluated in a specially designed test box Pairs of matched rats either SHR and NCR or renal hypertensive rats (RHR) and NCR were always used in these experiments The box consisted of two identical chambers sized

25 x 20 x 25 cm and separated by a wall into which two loud speakers were mounted and directed towards each chamber Sawdust was placed on the floor to make the environment as familiar as possible Three different types of alerting stimuli were used i e sudden flashes of bright light which was the weakest type of stimulus loud noise and vibrations which latter proved to be the strongest alerting stimulation Thus above the two chambers two parallel-coupled 100 W lamps were mounted which could deliver flashes of variable intensity and at random intervals The loud noise of variable intensities and frequencies was delivered to the two chambers through their loud speakers at irregular intervals The test box was mounted on four strings and could suddenly be vibrated by a motor driven uncentered wheel under the box

After tail artery cannulation the two rats were left undisturbed in the test box for 60 minutes in the sound isolated semidark laboratory before they were exposed to any alerting stimuli Via long polyethylene catheters connected to Statham pressure transducers placed outside the box at the level of the rats their mean arterial pressures and heart rates were continuously followed on a Grass polygraph using a tachygraph that was triggered by the systolic pressure rise Each alerting stimulus lasted for 30 seconds with intervals of 10 - 15 minutes between stimulations

When drugs were administered they were given via the arterial cannula dissolved in 0 4 - 0 5 ml of saline Partly due to the local anesthesia the animals appeared in general to be quite undisturbed by the tail artery cannulation and sat calmly in the box chambers after an initial exploration licking their fur and sometimes even sleeping However occasionally rats were somewhat excited particularly SHR and expecially those which had earlier been kept socially isolated Not infrequently they explored the thin catheters and bit them off thus causing blood loss In these cases the experiment was discarded Characteristically this never happened with NCR which in general were more peaceful

Hindquarter perfusion procedures

In studies II III and IV the isolated hindquarter vascular beds of pairs of SHR and NCR were simultaneously perfused in order to evaluate the structural design of the resistance vessels A detailed methodological description of this technique is given in study III as well as by Lundgren (1974) and Weiss (1974) and will therefore only be briefly outlined in this connection

Perfusion medium

Since the paired isolated hindquarters were perfused with a non-recir-

culating plasma substitute neurohormonal or blood borne interferences with vascular tone were eliminated As perfusate was used oxygenated Tyrode solution containing in mM/l: NaCl 136 9 KCl 2 7 $CaCl_2$ 1 9 $MgCl_2$ 1 1 $NaHCO_3$ 11 9 NaH_2PO_4 0 4 and glucose 5 6 to which 3 per cent (study III) or 4 per cent (study II and IV) of Ficoll (a synthetic polymer of sucrose and epichlorohydrin Pharmacia AB Uppsala Sweden) was added as a colloid substitute Depending on the limited supply different batches of Ficoll had to be used and the molecular weights were either 80 000 (study III), 70 000 (study II) or 47 000 (study IV) The temperature of the perfusate reaching the preparation was 35°C pH was 7 35 and osmotic pressure around 300 mOsm/l The viscosity of the perfusate determined in a Wells Brookfield Micro viscosimeter varied between 1 1 and 1 3 depending on whether 3 or 4 per cent Ficoll was used

However these variations in perfusate composition between the different studies will not interfere with the comparison between SHR and NCR since all results are based on differences in paired experiments where occasional variations in perfusate composition or temperature will influence SHR and NCR to an equal extent When comparing the mean values of study II and III such interferences as well as the use of the combined skin and muscle preparation of both males and females in study III probably accounts for the different absolute values obtained Although colloids were added to the perfusate edema formation occasionally occurred towards the end of the experiment This was especially noted in study IV where the maximally dilated vascular beds were exposed to high transmural pressures and in later phases to continuous exposure to barium ions which intensely constrict also the postcapillary resistance vessels thereby increasing capillary pressure markedly (Folkow et al 1974)

Technical arrangements

A schematic illustration of the experimental setup is given in Fig 1 The technique which was first introduced in study III has been slightly modified afterwards but only concerning details Thus the perfusion pressures which originally were measured via a side tube in the wide aortic cannula were later recorded in the caudal artery thereby avoiding any possible interferences in terms of a slight pressure drop along the short aortic cannula end However in study III flow was kept constant throughout the experiment and the minor flow resistance offered by the aortic cannula was recorded after each experiment and subtracted from the presented values By cannulating the tail artery as was done in studies II and IV the main part of the tail circulation was eliminated Since this vascular bed like that of the feet represents mostly skin a

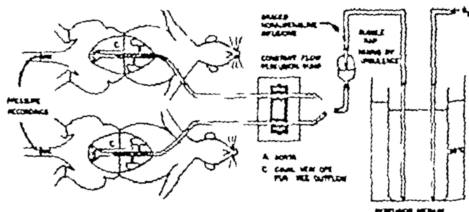


Fig 1 Schematic illustration of the experimental arrangements during paired hindquarter perfusion used in studies II III and IV The oxygenated 38°C warm perfusate passes first via a common tube into which e g graded noradrenaline doses can be infused prior to a mixing chamber The tube emerging from the mixing chamber branches into two parts which after passing through a double Harvard perfusion pump are connected to the two animals via their respective aortic cannulas (A) After passing the hindquarter vascular beds the perfusate leaves freely via the cut inferior caval veins (C) Perfusion pressures are measured via the tail (caudal) arteries

---

more pure muscle preparation was obtained in studies II and IV by lacing tight ligatures around the feet and tail

Further improvement of the technique for evaluating the resistance vessel design was introduced by performing pressure-flow recordings during maximal dilatation which was first secured by repeated injections of papaverine For each individual experiment pressure flow curves were constructed and the resistance at maximal dilatation at different distending pressures and flow rates could thereby be calculated Thus by presenting resistance at maximal dilatation at the higher flow rate of 30 ml/min x 100 g as in study II the impact of differences in vascular distensibility between SHR and NCR will also be revealed (see paper IV)

Then graded vasoconstrictions were induced by stepwise increases of noradrenaline infusion during constant flow perfusion until maximal pressor responses to NA had been induced In study II a final addition of supramaximal amounts of barium chloride and vasopressin was added to secure a definitely maximum contractile effort of all vessels contributing to flow resistance Then resistance curves were plotted for each animal with log NA dose on the abscissa and the pressor (resistance) responses on the ordinate (cf study III)

Fig 2 Schematic illustration of the anterograde perfusion of isolated heart preparations used in study V

Isolated heart perfusion

The perfusion technique used in paper V was originally developed by *Morgan et al (1965) and modified by Isaksson (1972) for studying cardiac metabolism* In contrast to the Langendorff preparation this method allows the heart to maintain its pumping function in vitro, since the perfusate enters the heart via the cannulated left atrium and leaves the heart via the aorta For details concerning the perfusion procedure see paper V and Isaksson (1972) A schematic illustration of the perfusion apparatus is given in Fig 2 By using this anterograde perfusion of an isolated heart changes in cardiac work can be evaluated since the load on the heart can be varied by increasing either the preload or the after load The preload was varied by changing the level of the atrial bubble trap (see Fig 2) The afterload was basically determined by the height *of a hydrostatic pressure set at 70 cm H_2O above the heart but during* ventricular expulsion the afterload was somewhat raised depending on the

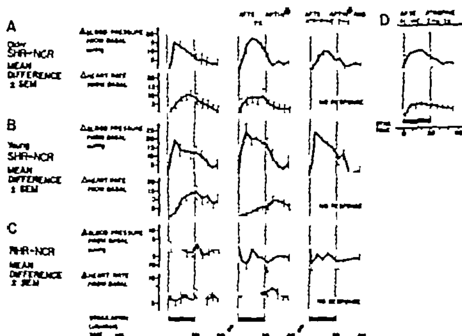


Fig 3 Mean differences between 7 month old SHR and NCR (A) between young SHR and NCR (B) and between RHR and NCR (C) concerning heart rate and blood pressure responses during 30s exposure to vibrations and the subsequent 30s poststimulatory period From left to right are shown the responses before any drugs are given after administration of a β-adrenergic blocking agent (Aptin ®) and after both Aptin ® and atropine istration The far right section shows the situation when SHR and NCR are given atropine alone (D) The differences in blood pressure between hypertensive and normotensive animals in each group are based on the mean of the differences obtained in each paired experiment as calculated each third second Likewise the differences in heart rate are first expressed as the mean difference in each pair of hypertensive and normotensive animals given here as percentual change from baseline However when given as absolute changes in heart rate the differences are equally significant The mean of all these differences are presented as curves with SE indicated as vertical bars

1974) showed about equal blood pressure increases as NCR Therefore the balance between cardiac output and total peripheral resistance seems to be about equal in RHR and NCR although both vasoconstrictor and vasodilatator responses are likely to be exaggerated in RHR due to the previously mentioned structural vascular changes On the other hand in SHR there were clear signs of an increased sympathetic discharge in response to alerting stimuli also involving the heart

Other things being equal this is likely to change the balance between cardiac output and total peripheral resistance in such a direction as to cause a more pronounced increase of arterial pressure in SHR than in NCR or RHR

The relative roles of sympathetic and vagal modulations of heart rate were judged in study I by comparing the heart rate changes after adrenergic β-receptor blockade or after atropine After β-blockade the heart rate of SHR fell significantly more than that of NCR or RHR With atropine administration alone heart rate increased more in NCR than in SHR These drug effects thus suggest an increased sympathetic but a reduced vagal discharge to the heart in SHR when compared to NCR and RHR and this difference was present also when the animals were not exposed to the alerting stimulations However the foreign environment may well imply a certain degree of stimulus and the observed differences in autonomic control between SHR and NCR might therefore be smaller during complete rest In any case the intrinsic heart rate i e that after total nervous blockade to the heart by administration of both a β-blocker and atropine was slightly though significantly lower in SHR than in NCR Therefore the mentioned shift in neurogenic cardiac control in SHR even if present during complete rest does not necessarily imply any higher resting heart rate in SHR than in NCR

The tachycardia responses to the alerting stimuli were not only more intense in SHR but also were more consistently elicited than in NCR and RHR in which one third of the responses were in the direction of bradycardia This bradycardia response to alerting stimuli was not primarily a reflex adjustment to the pressure rise but must have been centrally elicited since it slightly preceded the pressure rise in most cases Consequently NCR and RHR responded more frequently than SHR to these types of stressful stimuli with an autonomic pattern that includes vagal activations while SHR responded more consistently with sympathetic excitation combined with vagal suppression After atropine alone all animals responded with tachycardia to the alerting stimuli used but SHR responded most intensely This illustrates that the above mentioned bradycardia in NCR RHR was purely vagal in origin Furthermore when clearcut tachycardia responses occ red which were associated only with an increased cardiac adrenergic discharge these responses were more in tense in SHR than in NCR As mentioned above also the blood pressure responses to the alerting stimuli were more pronounced in SHR particularly in the young animals where the average pressure rise was 4–5 times larger and also tended to be more prolonged than in either NCR or RHR (see Fig 3)

When taken together these results suggest an increased central reactivity in SHR for eliciting excitatory cardiovascular responses during alertness and mental stress in the direction of defence reactions This

central hyperreactivity and apparent prevalence for sympathicotonic patterns in SHR evidently reflect a genetically linked difference from NCR and is not dependent on the high blood pressure per se Thus it was evident also in the young prehypertensive SHR while RHR which lack genetic predisposition to high blood pressure *responded* like NCR

Besides displaying more intense and somewhat more prolonged defence reactions SHR also responded more frequently with pressure and heart rate increases to the weakest alerting stimuli used indicating a lower threshold than in NCR for eliciting defence reactions in response to environmental stimuli Since the normal environment of rats or any species probably offers frequent situations calling for enhanced alertness the above mentioned difference in central reactivity is likely to result in more frequent intense and prolonged pressure increases in SHR than in NCR Such transiently enhanced neurohormonal discharges during daily life must imply an increased average pressure load on the SHR cardiovascular system already in prehypertensive or *early phases of hypertension This is likely to trigger a gradual structural* cardiovascular adaptation (see below) Such functional and structural factors tend to potentiate each other in a way which appears to be crucial for establishing a chronic hypertensive state

In order to test the relevance of such a proposed sequence of events SHR were in study II kept isolated from the age of weaning for 6 months in order to eliminate such environmental stimuli that are inherent in social confrontations Although their cardiovascular [illegible] to standardized alerting stimuli were at least as vivid as in [illegible]lated control SHR the development of hypertension was *delayed* [illegible] surpressed in these isolated SHR compared to age matched control SHR In contrast the blood pressure level of similarly isolated NCR did not differ from their unisolated controls (see Fig 4) Moreover the structural cardiovascular adaptation was correspondingly less pronounced in the isolated SHR compared with the control SHR (see also below) Therefore these findings indicate an important interaction between a genetically linked hyperreactivity concerning cardiovascular pressor responses in SHR and trivial stimuli per se, inherent in their normal environment Together such functionally interacting elements will contribute as efficient trigger mechanisms to the gradual development of structural cardiovascular adaptation

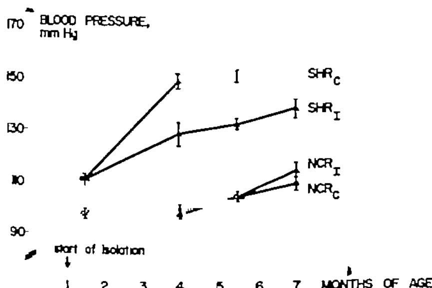
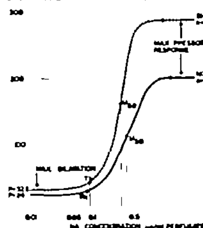


Fig 4 Levels of mean arterial pressure as measured intraarterially during resting awake conditions (± SE) for control SHR (SHR$_C$ n = 10), isolated SHR (SHR$_I$ n = 9) isolated NCR (NCR$_I$ n = 10) and control NCR (NCR$_C$ n = 10) followed from the age of 1 5 months up to 7 months of age

Structural adaptation of the resistance vessel in SHR

Study III - The evaluation of changes in the design of the SHR resistance vessels was carried out by quantitative measurements of the pressure responses during constant flow perfusion of the hindquarter vascular beds in pairs of SHR and NCR This hemodynamic estimation of resistance vessel design was based on the analysis of the NA dose response curves in SHR and NCR Starting from maximal dilatation, the degree of resistance vessel constriction was gradually increased until the maximal pressor response of the resistance vessels was reached which represents their maximal contractile strength This technique was developed in study III and utilized also in study II

In agreement with earlier findings in hypertensive man a [illegible] resistance was demonstrated in SHR compared to NCR already at smooth muscle relaxation The NA threshold dose" was defined [illegible] NA concentration in the perfusate which increased resistance [illegible] above that at maximal dilatation which roughly corresponds [illegible] cent shortening of completely relaxed vascular smooth muscle [illegible] NCR did not differ in this respect which showed that SHR [illegible]



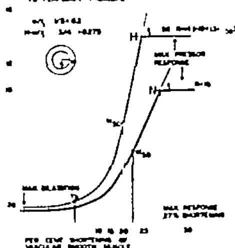


Fig 5 Left average resistance curves for SHR and NCR based on the results of 15 paired experiments from the state of maximal dilatation to maximal pressor (resistance) responses to noradrenaline Right: mathematically deduced resistance curves for two hypothetical resistance vessels H and N where H differs from N only in that its media thickness is increased 30 per cent encroaching upon its lumen even at complete smooth muscle relaxation w/r_i = ratio of wall thickness to internal radius

smooth muscle was not hypersensitive to NA which is in agreement with findings on isolated strips of vessels (e g Spector et al 1969 Hallbäck Lundgren and Weiss 1971) However both the steepness of the resistance curve and the maximal pressor response to supramaximal doses of vasoconstrictor agents were increased in SHR i e the hypertensive resistance vessels exhibited exaggerated luminal reductions and an increased maximal contractile strength compared with NCR When taken together these findings suggest an increased media thickness partly encroaching upon the lumen even during maximal dilatation but with a largely normal smooth muscle sensitivity to the adrenergic transmitter

This interpretation of the experimental resistance curves (Fig 5 *left part) was supported by a comparison with computed resistance curves* for two hypothetical resistance vessels one normotensive (N) and one hypertensive (H) N and H were assumed to be identical except for a 30 per cent thicker media in H which correspondingly reduced the lumen at complete relaxation Thus while the wall/lumen ratio at maximal dilatation was set to 1/5 for N (cf van Citters 1966) it was 1 3/4 7 for H (Fig 5 right part) For construction of the experimentally ob-

tained resistance curves in SHR and NCR the NA doses are plotted in a log scale along the abscissa To give a comparable scale on the abscissa for the N H resistance curves the S-shaped relationship between log NA and shortening of vascular strips was utilized From this relationship the per cent smooth muscle shortening could be plotted along the abscissa in due proportion to log NA dose (Fig 5 right part) Furthermore the maximal contractile strength of N was arbitrarily set to 10 times the resistance (or pressure) at maximal dilatation With an equal ability for tension development per unit contractile element in N and H but with 30 per cent thicker media in H its maximal strength is 30 per cent greater than in N When a correction according to Laplace s law for the smaller radius in H is taken into account (see Fig 5 right part) a 40 per cent higher maximal pressor response is obtained in H compared with N

NA sensitivity is assumed to be equal in N and H in accordance with findings on arterial strips from SHR and NCR (Clineschmidt et al 1970 Hallbäck Lundgren and Weiss 1971) Furthermore the contractions are considered to be initiated from the outermost media layer since the adrenergic neuroeffector junctions in precapillary resistance vessels are concentrated to this site (e g Norberg and Hamberger 1964) Also the great majority of α-receptors appear to be situated here judging from in vitro responses of vascular smooth muscle to exogenous NA (Johansson et al 1970) When applied to the model vessels it implies that inner wall layers become displaced towards the lumen when contraction occurs and the thicker the wall the more exaggerated the resistance increase will be It is therefore clear that a resistance vessel design like that in H will proportionally raise the baseline i e the resistance at maximal dilatation cause an increased steepness of the resistance curve and raise the maximal pressor response at normal functional characteristics per se of the effector cells in relation to N It is seen from Fig 5 that the resistance curves of the two model vessels in their mutual relationships closely mirror those of the NCR SHR resistance vessels

In fact should the altered characteristics of the SHR resistance curve be ascribed to other factors one must not only assume a simultaneous involvement of several different changes but also disregard all hemodynamic consequences of the welldocumented wall thickening in hypertensive precapillary vessels For example an increased muscle sensitivity to NA would only cause a parallel displacement of the resistance curve to the left but not alter its configuration An increased tension development per unit contractile element being per se unlikely as the contractility of larger SHR arteries is if anything decreased (e g Spector et al 1969) cannot explain the increased resistance at maximal dilatation A reduced size or number of resistance vessels but with an unchanged wall/lumen ratio could as suggested by Hutchins

and Dornell (1974) explain the increased resistance at maximal dilatation but certainly not the increased maximal contractile strength of the SHR resistance vessels which has been tested in model experiments (unpublished results) Likewise increases of wall/lumen ratio by addition of noncontractile elements or water logging (Tobian and Binion 1952) cannot explain the markedly increased maximal contractile strength of the SHR resistance vessels since this calls for a larger mass of contractile elements

Therefore the present experimental data (study III) clearly indicates that the SHR resistance vessels exhibit a hemodynamically most important increase in wall/lumen ratio which is mainly the result of a relative thickening of the muscle sheath This changed resistance vessel design may in fact explain their hyperreactivity and ability to maintain a raised resistance which does not necessitate any tonic increase of smooth muscle activity Furthermore in a more recent study it could be demonstrated that only the precapillary resistance vessels in SHR show exaggerated resistance responses and signs of altered design while capillaries and postcapillary vessels appear largely normal in dimensions design and reactivity (Folkow et al 1974)

Study IV - A partly different approach for exploring the altered vascular design in SHR was used in study IV Here the distensibility of the resistance vessels was studied in SHR and NCR both at maximal dilatation and at increasing levels of smooth muscle contraction Earlier studies demonstrating reduced vascular distensibility in hypertension have mostly dealt with large arteries (e g Feigl Peterson and Jones 1963 Greene et al 1966) whereas the present study was designed to study the true resistance vessels Paired hindquarter perfusions were performed in a similar way as in studies II and III During maximal dilatation or at stable levels of smooth muscle activity obtained by suitable concentrations of $BaCl_2$ or NA in the perfusate flow was altered in a randomized fashion and the resulting pressure changes were recorded According to Poiseuille s law resistance (R) varies inversely with the fourth power of the internal radius and hence the internal radius of the average resistance vessel was arbitrarily expressed as $\sqrt[4]{1/R}$ The distensibility of the resistance vessels was thus compared in SHR and NCR by relating the per cent increase of internal radius for a 10 mm Hg change in average distending pressure which was calculated as the mean of the arterial and venous pressures

During maximal dilatation when nonmuscular wall elements are likely to offer the main resistance to the distending pressure the distensibility of the SHR resistance vessels was significantly lower (some 11 per cent) than that of the NCR ones During smooth muscle activity the shortened actomyosin complexes offer the main resistance to distension

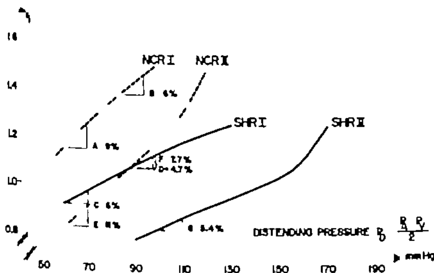


Fig 6 The relationship between average distending pressure (P_D) and calculated internal radius (r_i) for the average resistance vessel in 3 representative pairs of SHR and NCR Curves labelled I represent the situation when flow resistance was kept increased about 3 times above that at maximal dilatation induced by barium chloride while curves labelled II represent a vascular tone approximately 5 times that at maximal dilatation Comparisons are made between NCR and SHR concerning resistance vessel distensibility (ΔR_i %/ΔP_D) at the same initial P_D (A and C) at the same initial r_i (E and G) and when both P_D and r_i are initially the same (F and D) See also text

since they tend to unload other wall elements (cf van Citters Wagner and Rushmer 1962) Therefore the distensibility of the SHR NCR resistance vessels was also compared during stable levels of vascular smooth muscle tone which largely corresponded to those present in resti g skeletal muscle in vivo During smooth muscle contraction the difference in resistance vessel distensibility between NCR and SHR increased about threefold compared with the difference seen during maximal dilatation

When comparing the distensibility of the NCR SHR resistance vessels distending pressure smooth muscle activity and internal radius should ideally all be kept as equal as possible in the two circuits However this is impossible because of the increased reactivity of the SHR precapillary resistance vessels For such reasons resistance vessel distensibility was first compared at equal smooth muscle activity and internal radii but at different distending pressures (E and G Fig 6) Then the comparison was repeated when smooth muscle activity and distending pressure were equal but at different internal radii (A and C Fig 6) Lastly a

comparison was made when both distending pressure and internal radii were the same in NCR and SHR (F and D Fig 6) in which case less $BaCl_2$ (or NA) had to be given to the hyperreactive SHR precapillary resistance vessels thus implying a lower level of vascular smooth muscle activity in SHR

The compiled results of 14 experiments show that during contraction the SHR resistance vessels are some 35-45 per cent less distensibel than the NCR ones irregardless of the mode for comparison Thus these results strongly suggest that particularly the media component of the SHR resistance vessels is increased in bulk Especially comparisons like that shown as F D in Fig 6 definitely exclude that the difference in behavior and reactivity between SHR and NCR resistance vessels is merely due to some difference in smooth muscle activity

Furthermore these results also illustrate the raised contractile strength of the SHR resistance vessels here reflected by their decidedly higher yield pressures when exposed to such high distending pressures that the contracted vascular smooth muscles were forced to yield This was revealed as an abrupt and steep decrease in resistance in Fig 6 shown as an increase of calculated internal radius when the distending pressure exceeded a critical level which was considerably higher in SHR than in NCR It follows that these results concerning the distensibility of the resistance vessels lend further strong support to the results and conclusions in study III

Study II - The extent of change in structural vascular design seems to be largely proportional to the increased arterial pressure level in SHR indicating a close correlation between pressure load and structural adaptation of the resistance vessels Studies in renal hypertensive rats show that the duration and extent of blood pressure increase determine the degree of structural vascular adaptation which seems to be fully established within 3-4 weeks in young animals (Lundgren 1974) Furthermore a local or general reduction of the functional pressure load prevented the development of structural changes in the SHR resistance vessels or caused regression of already established changes (Folkow et al 1971 Weiss 1974)

Towards such a background the resistance vessel design was hemodynamically analyzed in a group of SHR which from weaning had been isolated from social confrontations This change of environment is likely to reduce the frequency and extent of intermittent increases in central neurohormonal discharge to the cardiovascular system In SHR the extent of these discharges seems to be exaggerated for genetical reasons (study I) The hemodynamic analysis of the hindquarter vascular bed in these animals was carried out at 7 months of age after prolonged isolation and gave the following results The slope of the resistance curves reflecting

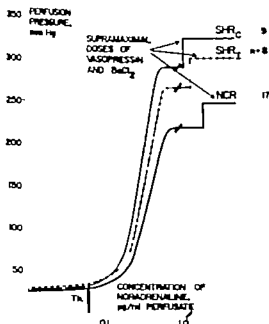


Fig 7 Average resistance curves of constant flow perfused hindquarter vascular beds from control SHR (SHR_C) SHR kept isolated from social contacts for 5 1/2 months (SHR_I) and paired NCR The curves show the mean resistance (pressure) changes from maximal vasodilatation to maximal smooth muscle activation as induced by graded noradrenaline infusions and finally by adding supramaximal doses of vasopressin and $BaCl_2$ Th indicate the threshold NA dose

---

the wall/lumen ratio of the resistance vessels and the maximal pressor response reflecting their contractile strength were both significantly reduced in isolated SHR compared to the unisolated control SHR Resistance at maximal dilatation did not differ significantly (Fig 7) Also the structural adaptation of the left ventricle was proportionally less pronounced in the isolated SHR compared with the control SHR as judged by their significantly lower per cent left ventricular weight of body weight

These results therefore illustrate the important consequence of functionally induced differences in average arterial pressure level for the design of the cardiovascular high-pressure sections Thus any reduction in environmentally induced neurogenic pressor influences which in SHR seems to be amplifi d due to a central autonomic hyperreactivity to psychogenic stimuli implies a reduction of the average functional pressure load and hence a less pronounced structural adaptation with a corre-

spondingly lower resting arterial pressure Thus the potential vicious circle inherent in the interaction between functional excitatory influences and secondary structural changes in SHR can to some extent be counteracted by limiting such exogenic stimuli which tend to reinforce the inherent hyperreactivity of higher autonomic centres

Structural adaptation of the heart in SHR

The establishment of left ventricular hypertrophy seems to take place very early in SHR and increases in extent as hypertension proceeds (e g Sen et al 1974 Farmer et al 1974) In the present study (V) the consequence of this structural cardiac adaptation for the Frank Starling relationship was investigated in isolated perfused heart preparations from young and adult SHR The results show that the hypertrophied hearts from adult 7 month old SHR require a higher diastolic filling pressure to produce the same left ventricular stroke volumes or stroke work than hearts from age matched NCR when compared at the normal range of left ventricular filling pressure between 5 and 10 cm H_2O (Fig 8 left part) At higher filling pressures this difference was no longer apparent since the NCR Starling curve tended to level off at filling pressures when that of the SHR ones was still ascending

This shift to the right of the SHR Starling curve could not be ascribed to any compensatory luminal change because end diastolic volumes were largely the same as in age matched NCR compared at equal filling pressures These latter measurements were performed on isolated SHR and NCR hearts arrested by and filled with ice-cold Krebs solution during exposure to different filling pressures while left ventricular volume (weight) was continuously recorded The obtained curves relating pressure and calculated intra ventricular volume did not differ significantly between SHR and NCR although the percentual change in left intra ventricular volume per unit change in pressure tended to be somewhat lower in SHR

The measured end-diastolic volumes and the wet weight of the left ventricles also allowed for a calculation of left ventricular geometry and wall/lumen ratio at different end diastolic filling pressures by assuming the ventricle to be a sphere As a result of the thicker wall of the SHR left ventricle the elongation of the outer and mean circumferences representing the length of the outer and mean myocardial elements were shown to be significantly smaller in SHR than in NCR upon equal increases in filling pressure Moreover calculations of wall tension per unit tissue mass which determines the degree of stretch on the average myocardial fibre (Starling 1918) revealed that the average muscle fibre of the SHR left ventricle was exposed to a significantly

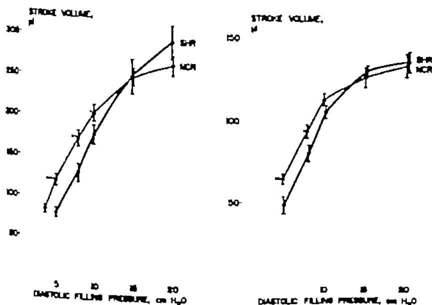


Fig 8 Relationship between diastolic filling pressure and stroke volume (Frank Starling relationship) for 30 week old SHR and NCR (left part) and for 6 week old SHR and NCR (right part)

lower tension at a given end-diastolic filling pressure compared with NCR In other words the more the left ventricular wall/lumen ratio is increased by hypertrophy the less pronounced is the average myocardial diastolic prestreching for any given increase in filling pressure even though left ventricular distensibility as judged by the luminal changes is not necessarily changed

Comparisons of the Frank-Starling relationship of hearts from NCR and SHR 6 week old and representing early phases of hypertension were also performed Already at this age mean arterial pressure and left ventricular weight/body weight ratio were significantly increased in SHR although far less so than in adult SHR In the lower range of diastolic filling pressures (5-10 cm H_2O) the stroke volumes were also significantly reduced in these young SHR compared to age matched controls although to a less extent than in adult SHR (Fig 8 right part) However the stroke work i e the product of left ventricular output and the pressure built up during ejection was then only slightly decreased in SHR and at higher filling pressures (15-20 cm H_2O) the SHR left ventricles exhibited significantly greater stroke work than the NCR ones This indicates a elatively greater flow acceleration induced by the SHR left ventricle during ejection since stroke volumes and heart rates at

these higher filling pressures were largely equal in SHR and NCR Thus maximal cardiac work performance of the young SHR was significantly greater than that of matched controls which probably reflects the increased bulk of cardiac muscle in SHR already at this age In older SHR the maximal left ventricular work performance could not be evaluated since their hearts had not reached the peak of the Starling curve at the highest filling pressures tested

At a diastolic filling pressure of 8 cm H_2O the young SHR having a 40 per cent increase in the ratio left ventricular dry weight to body weight displayed a 10–13 per cent reduction in stroke volume compared with age matched NCR Whereas the adult SHR with a 80 per cent increase in left ventricular weight/body weight showed a 20–25 per cent reduction in stroke volume compared with matched NCR Thus with the progression of hypertension the Frank Starling relationship of the progressively more hypertrophied left ventricle becomes reset so that a higher end diastolic filling pressure is required for the same stroke volume as in matched normotensive controls

When applying these findings to the in vivo situation where neurogenic influences are superimposed on the heterometric autoregulation of cardiac performance (Sarnoff and Mitchell 1962) the maintenance of equal left ventricular stroke volumes in NCR and in SHR with established hypertension would call for a somewhat increased sympathetic drive to the capacitance side and/or to the heart in SHR Dependent on the age-linked progression of hypertension and left ventricular hypertrophy the presence of an increased sympathetic influence may in young SHR result in an increased stroke volume and cardiac output compared with controls This hemodynamic pattern often characterizes e g labile hypertension in man On the other hand for older SHR with more developed cardiac hypertrophy equally increased sympathetic discharge may imply a largely normalized stroke volume and cardiac output which is the general finding in established hypertension in man

GENERAL DISCUSSION

As outlined in Results and comments the hyperreactivity of spontaneously hypertensive rats (SHR) to alerting or stressful stimuli is of central origin It reflects a response pattern where the cardiac acceleration involves both an increased sympathetic and decreased vagal tone which is characteristic of the hypothalamic defence reaction (study I) These centrally induced cardiovascular responses to the stressful stimuli utilized were not only to be quantitatively exaggerated in SHR but also to some extent qualitatively different in SHR and NCR Thus neurogenic adjustments characterizing the defence reaction seem to be dominating in SHR while NCR exhibits some variability between individuals with initial bradycardia responses occurring in about 35 per cent of the cases Such individual variations concerning both changes in heart rate and skeletal muscle vasodilatation have also been observed in awake normotensive cats when exposed to different types of emotional excitement (Zanchetti Baccelli and Mancia 1971) It appears as if normally a spectrum of differently balanced autonomic response patterns are available where any one of them can be engaged depending on individual circumstances and type of stressful stimuli

The central hyperreactivity to alerting stimuli in SHR appears to have largely the same consequences for the cardiovascular system as when normotensive rats are exposed to environmental stimuli that are enhanced in extent frequency and duration Several reports have demonstrated that prolonged exposure to stressful or socially conflicting stimuli can induce more or less persistent increases in arterial pressure even in genetically normotensive laboratory animals (Henry Meehan and Stephens 1967 Herd et al 1969 Smookler et al 1973 Alexander 1974) In the present study (II) a prolonged elimination of di ect social confrontations (even though some contacts with the other animals were still present by way of e g sound and smell signals) partly surpressed the development of hypertension in SHR However they still exhibited the characteristically exaggerated cardiovascular responses to standardized alerting stimuli Thus their environmental situation was aimed to be a contrast to that of genetically normotensive animals when exposed to prolonged intensification of environmental stimulations

It therefore seems obvious that enhanced and often repeated cardiovascular pressor responses to emotional stimuli whether induced by an inherent central hyperreactivity to such stimuli as in SHR or by increasing their intensity duration and frequency in normal animals can be of great importance for the initiation of hypertension However the SHR hypertension which is generally acknowledged to have a polygenic background (cf Okamoto 1972) seems to be more stabile and pronounced

these higher filling pressures were largely equal in SHR and NCI maximal cardiac work performance of the young SHR was signific greater than that of matched controls which probably reflects th creased bulk of cardiac muscle in SHR already at this age In ol SHR the maximal left ventricular work performance could not be ted since their hearts had not reached the peak of the Starling cu the highest filling pressures tested

At a diastolic filling pressure of 8 cm H_2O the young SHR a 40 per cent increase in the ratio left ventricular dry weight to weight displayed a 10-13 per cent reduction in stroke volume co with age matched NCR Whereas the adult SHR with a 80 per cent crease in left ventricular weight/body weight showed a 20-25 per reduction in stroke volume compared with matched NCR Thus w the progression of hypertension the Frank-Starling relationship of th progressively more hypertrophied left ventricle becomes reset so tha higher end-diastolic filling pressure is required for the same stroke as in matched normotensive controls

When applying these findings to the in vivo situation where n genic influences are superimposed on the heterometric autoregulatic of cardiac performance (Sarnoff and Mitchell 1962) the maintenanc of equal left ventricular stroke volumes in NCR and in SHR with est lighed hypertension would call for a somewhat increased sympathetic drive to the capacitance side and/or to the heart in SHR Depender on the age-linked progression of hypertension and left ventricular hy trophy the presence of an increased sympathetic influence may in yc SHR result in an increased stroke volume and cardiac output compare controls This hemodynamic pattern often characterizes e g labile h tension in man On the other hand for older SHR with more develop cardiac hypertrophy equally increased sympathetic discharge may impl a largely normalized stroke volume and cardiac output which is the general finding in established hypertension in man

hypertension (e g Eich et al 1966) It may be questioned whether this gradual transfer in hemodynamic balance is a reflection of some adaptive or/and compensatory process that also involves a change of cardiovascular design Here the gradual development of the well-known left ventricular hypertrophy and of structural changes also in other "high-pressure sections of the cardiovascular system i e arteries and precapillary resistance vessels (cf Folkow et al 1973 1974) is likely to be of importance

Such structural adaptations constituting a common mesenchymal response to changes in the mechanical stress applied (cf Liebow 1963 Rodbard 1970) mainly take the form of an increased bulk of contractile tissue at least in early stages In the resistance vessels such changes are generally associated with a luminal narrowing that is present even at complete smooth muscle relaxation as shown by hemodynamic studies in human essential hypertension (Folkow 1956 Folkow Grimby and Thulesius 1958 Conway 1963 Sivertsson 1970) Furthermore the present results in SHR (studies III IV) can hardly be explained by any other mechanism than by the presence of a relative increase in media thickness that partly interferes with the lumen of the precapillary resistance vessels These changes can be very rapidly established i e in 2-3 weeks after gross increases in pressure load as studied in young rats exposed to renal hypertension (Lundgren 1974) In addition sustained pressure reductions whether induced by antihypertensive treatment in SHR (Weiss 1974) or by renal artery declipping in renal hypertensive rats (Lundgren 1974) lead to a rapid and considerable egression of the structural changes in both resistance vessels and heart at least in young animals Also the present results (study II) illustrate the close correlation between average pressure load and cardiovascular structural adaptation when comparing ordinary SHR isolated SHR and NCR concerning the relationships between arterial pressure and degree of left ventricular hypertrophy and media hypertrophy in the resistance vessels

These adaptations in precapillary vessel design are closely balanced in extent by the change in pressure load may thus be denoted as a "structural autoregulation Thus it consitutes a morphological and consequently more slowly developed counterpart to the functional autoregulation confined to the precapillary resistance vessels (e g Folkow and Öberg 1961) which i caused by adj stments in smooth muscle activity to acute pressure changes Also the structural autoregulation implies a raised precapillary resistance in response to regional pressure increases and vice versa However since it is a matter of a change in vessel design the structural autoregulation is slower in development and does not necessitate any change in smooth muscle activity to maintain the altered resistance level

It appears as if the structural adaptation of the cardiovasc lar

high pressure sections may be somewhat more extensive in SHR than in genetically normotensive rats provoked into e g secondary renal hypertension (RHR) Thus when comparing the extent of alterations in left ventricular weight and resistance vessel design in RHR and SHR where both the degree and duration of hypertension were roughly the same, the structural changes appear to be more pronounced in SHR (cf Lundgren 1974) Moreover comparisons of resistance vessel design in NCR and in immunosympathectomized SHR point in the same general direction (Folkow et al 1972) In addition compared to NCR young prehypertensive SHR show an increased arterial incorporation of proline indicating a higher mesenchymal metabolic turnover in the SHR vasculature already at a stage where arterial pressures are largely similar in SHR and NCR (Yamori 1974)

When taken together such findings indicate that the SHR resistance vessels arteries and heart might for genetical reasons be more prone to adapt structurally to an increased pressure load than genetically normotensive rats Whether such a possible difference is inherent in cardiac and vascular structures per se or is mainly a consequence of a changed trophic influence of e g hormonal or neurogenic origin is unknown Whichever its exact background such an inherently increased tendency of the cardiovascular system to adapt structurally to increases in load may together with the previously mentioned central hyperreactivity in SHR form dominating genetic elements for the establishment of primary hypertension in SHR

As outlined above the structurally increased wall/lumen ratio in ...sive resistance vessels implies first a raised "baseline from which vasoconstriction is initiated and second exaggerated vasoconstric- and vasodilator responses for given changes in smooth muscle length While increased pressor responses are well documented in both human and SHR hypertension Increased depressor responses have mostly been regarded to reveal increased preexisting excitatory influences However it is clear e g from the resistance curves in Fig 5 that equal redu tions in smooth muscle activity in hypertensive and normotensive res tance vessels lead to exaggerated resistance reductions in the sive ones even if both start from the same level of smooth muscle ty

Interventions aiming at the elimination of sympathetic tone i SHR lead to a greater pressure fall in SHR than in NCR and ly low pressure levels are obtained after e g ganglionic pithing (Shibayama Mizogami and Sokabe 1971 Iriuchijima 1973 This has often been taken as strong evidence of an increased sy activity in SHR However arterial pressure is not only dependent peripheral resistance but also on cardiac output and their re well be altered to a different extent in SHR and NCR upon d

Furthermore the resistance reduction is likely to be exaggerated in SHR thanks to the presence of structural changes in its resistance vessels Careful measurements of cardiac output and mean arterial pressure before and after pithing in SHR and NCR with calculations of peripheral resistance show that the largely equalized pressures after pithing were the result of a higher resistance but a lower cardiac output in SHR compared with NCR (Albrecht et al 1975) The greater pressure fall in SHR was thus determined in part by a greater resistance reduction which probably reflects their exaggerated resistance responses as illustrated in Fig 5 However in part it was also due to a percentually greater reduction of cardiac output The presence of a raised resistance even after complete denervation when the marked pressure fall leads to nearly maximal vasodilatation in both SHR and NCR confirms earlier results showing a structurally determined generalized resistance increase in SHR (Folkow et al 1970)

The lower cardiac output in SHR after pithing may reflect structural differences in the low-pressure sections i e in the capacitance vessels or/and in the heart itself since cardiac performance upon denervation is determined mainly by the end-diastolic filling (cf Sarnoff and Mitchell 1962) The SHR capacitance vessels at least in the hindquarters seem to be largely unchanged compared with those of NCR (Folkow et al 1974) Therefore it seems more likely that the obvious left ventricular hypertrophy in SHR considerably influences the relationship between diastolic filling and cardiac performance which was explored in isolated perfused heart preparations from SHR and NCR in study V

In this study the Frank-Starling relationship of the isolated SHR left ventricle was shown to be displaced to the right of the NCR one in the lower physiological range of filling pressures This displacement was not a consequence of cardiac dilatation since end-diastolic volumes were about equal in the arrested left ventricles of adult SHR and NCR Furthermore when left ventricular compliance was determined as percentual change of intraventricular volume per unit change of end-diastolic pressure the SHR and NCR hearts did not differ significantly However it should be stressed that according to Laplace s law the thicker wall of the SHR left ventricle implies a lower tangential tension per unit wall layer for a given transmural pressure Consequently for equal filling pressures the stretching of the average muscle fibre becomes lower in the SHR left ventricle than in the NCR one

For such reasons the lower stroke volume in SHR appears to be primarily a consequence of the Frank-Starling law which implies that the end-diastolic fibre length rather than the end-diastolic pressure determines the stroke volume It is in agreement with such a view that stroke volumes were f~ ~ to be proportionally more reduced in adult

"high pressure sections may be somewhat more extensive in SHR than in genetically normotensive rats provoked into e g secondary renal hypertension (RHR) Thus when comparing the extent of alterations in left ventricular weight and resistance vessel design in RHR and SHR where both the degree and duration of hypertension were roughly the same, the structural changes appear to be more pronounced in SHR (cf Lundgren 1974) Moreover comparisons of resistance vessel design in NCR and in immunosympathectomized SHR point in the same general direction (Folkow et al 1972) In addition compared to NCR young prehypertensive SHR show an increased arterial incorporation of proline indicating a higher mesenchymal metabolic turnover in the SHR vasculature already at a stage where arterial pressures are largely similar in SHR and NCR (Yamori 1974)

When taken together such findings indicate that the SHR resistance vessels arteries and heart might for genetical reasons be more prone to adapt structurally to an increased pressure load than genetically normotensive rats Whether such a possible difference is inherent in cardiac and vascular structures per se or is mainly a consequence of a changed trophic influence of e g hormonal or neurogenic origin is unknown Whichever its exact background such an inherently increased tendency of the cardiovascular system to adapt structurally to increases in load may together with the previously mentioned central hyperreactivity in SHR form dominating genetic elements for the establishment of primary hypertension in SHR

As outlined above the structurally increased wall/lumen ratio in hypertensive resistance vessels implies first a raised baseline from which vasoconstriction is initiated and second exaggerated vasoconstric tor and vasodilator responses for given changes in smooth muscle length While increased pressor responses are well documented in both human and SHR hypertension increased depressor responses have mostly been regarded to reveal increased preexisting excitatory influences However it is clear e g from the resistance curves in Fig 5 that equal reductions in smooth muscle activity in hypertensive and normotensive resistance vessels lead to exaggerated resistance reductions in the hypertensive ones even if both start from the same level of smooth muscle activi ty

Interventions aiming at the elimination of sympathetic tone in SHR lead to a greater pressure fall in SHR than in NCR and about equally low pressure levels are obtained after e g ganglionic blockade or pithing (Shibayama Mizogami and Sokabe 1971 Iriuchijima 1973) This has often been taken as strong evidence of an increased s[illegible]thetic activity in SHR However arterial pressure is not only depen[illegible] peripheral resistance but also o[illegible]ac output and their rel[illegible] well be altered to a different [illegible] SHR and NCR upon d

Furthermore the resistance reduction is likely to be exaggerated in SHR thanks to the presence of structural changes in its resistance vessels Careful measurements of cardiac output and mean arterial pressure before and after pithing in SHR and NCR with calculations of peripheral resistance show that the largely equalized pressures after pithing were the result of a higher resistance but a lower cardiac output in SHR compared with NCR (Albrecht et al 1975) The greater pressure fall in SHR was thus determined in part by a greater resistance reduction which probably reflects their exaggerated resistance responses as illustrated in Fig 5 However in part it was also due to a percentually greater reduction of cardiac output The presence of a raised resistance even after complete denervation when the marked pressure fall leads to nearly maximal vasodilatation in both SHR and NCR confirms earlier results showing a structurally determined generalized resistance increase in SHR (Folkow et al 1970)

The lower cardiac output in SHR after pithing may reflect structural differences in the low-pressure sections i e in the capacitance vessels or/and in the heart itself since cardiac performance upon denervation is determined mainly by the end-diastolic filling (cf Sarnoff and Mitchell 1962) The SHR capacitance vessels at least in the hindquarters seem to be largely unchanged compared with those of NCR (Folkow et al 1974) Therefore it seems more likely that the obvious left ventricular hypertrophy in SHR considerably influences the relationship between diastolic filling and cardiac performance which was explored in isolated perfused heart preparations from SHR and NCR in study V

In this study the Frank-Starling relationship of the isolated SHR left ventricle was shown to be displaced to the right of the NCR one in the lower physiological range of filling pressures This displacement was not a consequence of cardiac dilatation since end-diastolic volumes were about equal in the arrested left ventricles of adult SHR and NCR Furthermore when left ventricular compliance was determined as per centual change of int aventricular volume per unit change of end-diasto- lic pressure the SHR and NCR hearts did not differ significantly How- ever it should be stressed that according to Laplace s law the thicker wall of the SHR left vent icle implies a lower tangential tension per unit wall layer for a given transmural pressure Consequently for equal filling pressures the stretching of the average muscle fibre becomes lower in the SHR left ventricle than in the NCR one

For such reasons the lower stroke volume in SHR appears to be primarily a consequence of the Frank-Starling law which implies that the end-diastolic fibre length rather than the end-diastolic pressure determines the stroke volume It is in agreement with such a view that stroke volumes were found to be proportionally more reduced in ad lt

SHR with their more advanced left ventricular hypertrophy than in young SHR Such structurally determined changes of the Frank-Starling relationship may also be of considerable importance in the in vivo situation For example cardiac output measurements in SHR of different ages indicate a decrease in stroke volume with the progression of hypertension (Pfeffer and Frohlich 1973) although in this case possible differences in superimposed neurohormonal influences must also be taken into account

Concerning the situation in man cardiac output is elevated or *normal in early hypertension and at the same time there are often signs* of an increased neurogenic activity to the heart (for ref see Julius and Schork 1971 and introduction) On the other hand established essential hypertension in man is characterized by left ventricular hypertrophy a largely normal cardiac output but an increased resistance Such a gradual transfer of the hemodynamics along with the development of hypertension may to a great extent be due to the presently studied interaction between functional initiating elements and the progressive structural adaptation of cardiovascular high-pressure sections

Thus SHR display a genetically determined central hyperreactivity for eliciting defence reactions in response to environmental stimuli Consequently such intermittent and neuro-hormonally mediated pressure increases tend to be more frequent pronounced and prolonged in SHR thereby raising the average blood pressure level Furthermore these pressor bursts are only poorly counterbalanced by the arterial baroreceptor reflexes since these are considerably surpressed by the defence reaction (cf Hilton 1963 Lisander 1970) Moreover also the long-term pressor regulating function of the kidneys (cf Guyton et al 1972) is likely to be overruled in connection with defence reactions partly because these reactions involve renal vasoconstriction and cause an acute reduction of urine formation (Feigl Johansson and Löfving 1964) and partly because of the concomitant renin-angiotensin-aldosterone release (e g Zanchetti and Stella 1975)

Along with the rise in average pressure within the high pressure cardiovascular sections the earlier mentioned structural autoregulation of the systemic precapillary resistance vessels is gradually initiated This structural autoregulation involves also the renal vascular bed (cf Folkow et al 1971) which in a more definite way will reset the long-term pressure regulating function of the kidneys since such a change in resistance vessel design implies that a higher preglomerular resistance can be maintained by a given smooth muscle activity Similarly by reducing the distensibility of larger arteries such structural adaptations seem to contribute also to the resetting of the arterial baroreceptors (Aars 1969) Also the left ventricular hypertrophy may be of great importance for the gradual shift in hemodynamics along with the development of hypertension since it tends to reduce left ventricular

stroke volume for a given filling pressure according to the present findings if no compensatory influences of e g neurogenic origin are superimposed

In other words these structural changes of the cardiovascular high-pressure sections have an inherent tendency to shift an early hyperkinetic circulatory state into one characterized by fairly stabilized resistance increase in association with a normal cardiac output and stroke volume Such a gradual shift in hemodynamics may start quite early in primary hypertension since the first clear signs of structural autoregulation of the resistance vessels can be found already at the age of 10–11 weeks in male SHR (unpublished) and the increase of left ventricular wall mass can be detected even earlier (e g Sen et al 1974) Furthermore already within 2 3 weeks after induction of renal hypertension in rats left ventricular hypertrophy and resistance vessel adaptation are largely completed (Lundgren 1974) Concerning man young conscripts to military service who exhibit modestly elevated mean arterial pressure show signs of mild structural autoregulation of their resistance vessels (Sivertsson Sannerstedt and Lundgren 1975) It is therefore possible that the raised resistance in relation to the increased cardiac output in the hyperkinetic state of early hypertension in part reflects an early involvement of such a structural adaptation

In general the present results illustrate how the establishment of chronic hypertension calls for the interaction of initiating functional elements and secondary structural changes where the latter appears to be the important common denominator and a prerequisite for chronic hypertension in general The initiating elements appear to be mainly constituted by central neurohormonal influences in the spontaneously hypertensive rat of the Okamoto strain (SHR) Concerning essential hypertension in man a similar train of events is likely to occur but here the initiating factors may be more heterogenous than in the pure-bred SHR

SUMMARY AND CONCLUSIONS

The present experiments were performed in order to elucidate the relative roles and temporal interaction between central neurogenic mechanisms and adaptive changes in cardiovascular design in primary hypertension as studied in spontaneously hypertensive rats (SHR) The results can be summarized as follows:

I SHR exhibited enhanced cardiovascular responses to acute alerting stimuli compared to normotensive control rats (NCR) Also after adrenergic β-receptor blockade SHR responded with enhanced and clearcut tachycardia which were abolished after atropine However there were no signs of any changed cardiac effector sensitivity in SHR in response to e g graded nerve stimulation This points to a central hyperreactivity in SHR for eliciting defence reactions which besides an increased sympathetic drive to the heart also includes a central inhibition of cardiac vagal tone Also young nearly prehypertensive SHR responded with enhanced defence reactions but not renal hypertensive rats This suggests that the central hyperreactivity in SHR is a genetically linked factor predisposing for hypertension and not a consequence of the high blood pressure *per se*

II When separated from social confrontations by means of isolation from the time of weaning the development of hypertension was delayed and surpressed in SHR compared with unisolated control SHR However the cardiovascular responses to acute psychological stress stimulations were equally pronounced in the isolated SHR as in SHR controls The results thus stress the importance of the interaction between environmental influences and inherently exaggerated cardiovascular responses to such stimuli for the development of hypertension in SHR

III By hemodynamic analysis of the hindquarter vascular beds of SHR and NCR an altered design of the resistance vessels in SHR was revealed Thus resistance responses to graded noradrenaline (NA) infusions resulted in exaggerated resistance responses in SHR which also exhibited an increased resistance at maximal dilatation and a raised maximal pressor response but an unaltered threshold sensitivity to NA These data suggest an increased media thickness encroaching upon the lumen of the resistance vessels also during complete smooth muscle relaxation This altered vascular design is according to later studies confined to the precapillary vascular sections and seems to be the result of a rapid local adaptation to increases in average pressure load and will by its mere presence tend to increase flow resistance and vascular reactivity in hypertension

IV Comparisons of the distensibility of the resistance vessels in SHR and NCR lent further strong support to the presence of an increased media thickness in the SHR vessels Thus they were clearly less distensible than the NCR ones both during maximal dilatation and particularly during equal levels of smooth muscle activity

V The hemodynamic consequences of an increased left ventricular wall thickness was investigated in isolated SHR and NCR hearts It was demonstrated that with the progression of hypertension and consequent left ventricular hypertrophy increased levels of end-diastolic filling pressure were required in the hypertrophied SHR left ventricles to produce the same stroke volumes as in NCR hearts The Frank-Starling relationship of the SHR left ventricles was shifted to the right in the lower physiological range of diastolic filling pressures as a result of the reduced diastolic compliance of the thickened wall Already in 6 week old SHR with only moderate left ventricular hypertrophy such a displacement of the Frank Starling relationship was clearly evident but it was proportionally less pronounced than in adult SHR

Compiled these results suggest that primary SHR hypertension may be initiated by a central hyperreactivity for eliciting defence reactions Environmental stimuli also seem to play a crucial role since the cardiovascular pressor response to the alerting stimuli of daily life presumably will be amplified due to the central hyperreactivity to such stimuli As a result of such intermittent pressor influences the average pressure load on the cardiovascular system will be raised in SHR thereby triggering structural cardiovascular adaptations of the high-pressure sections which seems to be a prerequisite for the establishment of a chronic hypertensive state It is further suggested that the progressive shift in hemodynamic balance between cardiac output and resistance along with duration and severity of hypertension is mainly due to the above mentioned structural cardiovascular adaptation Thus the structural autoregulation charac terizing the precapillary resistance vessels will contribute to the rise in peripheral resistance while the hypertrophic thickening of the left ventricle will tend to reduce cardiac output though both effects can be considerably modulated by superimposed neurogenic influences of central or reflex origin

ACKNOWLEDGEMENTS

I wish to express my deep gratitude to my teacher Professor Björn Folkow who introduced me into this field of research and encouraged me to start the present study to which his advice and invaluable help have greatly contributed

I wish to thank my co-workers Yen Lundgren and Lilian Weiss for most stimulating collaboration and many fruitful discussions To Olle Isaksson and Eddy Noresson I also want to express my thanks for stimulating cooperation

Excellent technical assistance and typing have been offered by Gunnel Andersson to whom I am most thankful Thanks are also due to Ulla Axelsson for skilful technical assistance and to Kerstin Lundgren and Lena Nimmerfors for typing the manuscript

The advice and help from Lars Stage in matters concerning statistical analyses and methodological problems are gratefully acknowledged as is the valuable help offered by the animal keeper Gösta Magnusson and the photographer Thomas Palm I am also much obliged to Dorothy Stage for scrutinizing the English text in this summary

This investigation was supported by grants from the Swedish Medical Research Council (No B75-14X-16-11A) from the Faculty of Medicine University of Göteborg and from the Swedish National Association against Heart and Chest Diseases

AB Hässle Mölndal Sweden generously covered part of the expenses for technical assistance Thanks are also due to AB Pharmacia Sweden for supply of Ficoll during the initial experimental series and to AB Abbott for gifts of Nembutal ®

Q

REFERENCES

AARS H Effects of arterial hypertension on aortic baroreceptor activity Oslo Universitetsforlaget 1969

ABRAHAMS V C S M HILTON and A ZBROZYNA Active muscle vasodilatation produced by stimulation of the brain stem: its significance in the defence reaction J Physiol (Lond) 1960 154 491-513

ALBRECHT I M HALLBACK S JULIUS Y LUNDGREN L STAGE, L WEISS and B FOLKOW Arterial pressure cardiac output and systemic resistance before and after pithing in normotensive and spontaneously hypertensive rats Acta physiol scand 1975 In press

ALEXANDER N Psychosocial hypertension in members of a Wistar rat colony Proc Soc exp Biol Med 1974 146 163-169

BACKER M Essential hypertension The birth of its concept two hundred years ago Angiology 1953 4 207 209

BELLO C T R W SEVY and C HARAKAL Varying hemodynamic patterns in essential hypertension Amer J med Sci 1965 250 24-35

BRIGHT R Tubular view of the morbid appearances in 100 cases connected with albuminous urine With observations Guy s Hosp Rep 1836 1 380-400

BROD J Haemodynamic basis of acute pressor reactions and hypertension Brit Heart J 1963 25 227-245

BROD J M CACHOVAN H U PIXBERG and D HARMJANZ Das Verhalten des Kapazitäts Blutbettes bei essentieller Hypertonie In Hypertension Ed A Distler and H P Wolff Georg Thieme Verlag Stuttgart 1974 pp 197 200

BROD J V FENCL Z HEJL J JIRKA and M ULRYCH General and regional hemodynamic pattern underlying essential hypertension Clin Sci 1962 23 339-349

BRUNJES S Catechol amine metabolism in essential hypertension New Engl J Med 1964 271 120-124

CELANDER O and B FOLKOW A comparison of the sympathetic vasomotor fibre control of the vessels within the skin and the muscles Acta physiol scand 1953 29 241 250

CLINESCHMIDT B V R G GELLER W C GOVIER and A SJOERDSMA Reactivity to norepinephrine and nature of the alpha adrenergic receptor in vascular smooth muscle of a genetically hypertensive rat Europ J Pharmacol 1970 10 45–50

COBBOLD A B FOLKOW O LUNDGREN and I WALLENTIN Blood flow capillary filtration coefficients and regional blood volume responses in the intestine of the cat during stimulation of the hypothalamic defence area Acta physiol scand 1964 61 467–475

COLEMAN T G H J GRANGER and A C GUYTON Whole-body circulatory autoregulation and hypertension Circulat Res 1971 28 and 29 Suppl 2 76–86

CONWAY J A vascular abnormality in hypertension A study of blood flow in the forearm Circulation 1963 27 520–529

CONWAY J Effect of propranolol in hypertensive rats Clin Sci Mol Med 1975 48 In press

DAVIES J O The control of renin release Amer J Med 1973 55 333–350

DEABLER H L E FIDEL R L DILLENKOFFER and S T ELDER The use of relaxation and hypnosis in lowering high blood pressure Amer J clin Hypnosis 1973 16 75–83

DE QUATTRO V and Y MIURA Neurogenic factors in human hypertension Mechanism of myth? Amer J Med 1973 55 362 378

DOYLE A E Vascular reactivity in human essential hypertension New Zeald med J 1968 67 295–305

DOYLE A E and J R E FRASER Vascular reactivity in hypertension Circulat Res 1961 9 755–758

EICH R H R P CUDDY H SMULYAN and R H LYONS Hemodynamics in labile hypertension A follow up study Circulation 1966 34 299 307

ELIASSON S B FOLKOW P LINDGREN and B UVNAS Activation of sympathetic vasodilator nerves to the skeletal muscles in the cat by hypothalamic stimulation Acta physiol scand 1951 23 333–351

ELLIS C N and S JULIUS Role of central blood volume in hyperkinetic borderline hypertension Brit Heart J 1973 35 450-455

ENGELMAN K B PORTNOY and W LOVENBERG A sensitive and specific double-isotope derivate method for the determination of catecholamines in biological specimens Amer J med Sci 1968 255 259-268

ENGELMAN K B PORTNOY and A SJOERDSMA Plasma catecholamine concentrations in patients with hypertension Circulat Res 1970 26 and 27 Suppl 1 141-146

EULER U S von Noradrenaline Springfield Ill Charles C Thomas Publ 1956

EWALD C A Ueber die Veränderungen kleiner Gefässe bei Morbus Brightii und die darauf bezüglichen Theorien Virchows Arch path Anath 1877 71 453-499

FARMER B B R A HARRIS W W JOLLY and W J VAIL Studies on the cardiomegaly of the spontaneously hypertensive rat Circulat Res 1974 35 102-110

FEIGL E B JOHANSSON and B LÖFVING Renal vasoconstriction and the defence reaction Acta physiol scand 1964 62 429-435

FEIGL E O L H PETERSON and A W JONES Mechanical and chemical properties of arteries in experimental hypertension J clin Invest 1963 42 1640-1647

FINCH L and G HAEUSLER Vascular resistance and reactivity in hypertensive rats Blood vessels 1974 11 145-158

FINKIELMAN S M WORCEL and A AGREST Hemodynamic patterns in essential hypertension Circulation 1965 31 356-368

FOLKOW B Structural myogenic humoral and nervous factors controlling peripheral resistance In Hypotensive Drugs Pergamon Press 1956 163 174

FOLKOW B Ce tral neuro-hormonal mechanisms in spontaneously hypertensive rats as compared with human essential hypertension Clin Sci Mol Med 1975 48 In press

FOLKOW B G GRIMBY and O THULESIUS Adaptive structural changes of the vascular walls in hypertension and their relation to the control of the peripheral resistance Acta physiol scand 1958 44 255-272

FOLKOW B M GURÉVICH M HALLBACK Y LUNDGREN and L WEISS The hemodynamic consequences of regional hypotension in spontaneously hypertensive and normotensive rats Acta physiol scand 1971 83 532-541

FOLKOW B M HALLBACK Y LUNDGREN R SIVERTSSON and L WEISS Importance of adaptive changes in vascular design for establishment of primary hypertension studied in man and in spontaneously hypertensive rats Circulat Res 1973 32 and 33 Suppl I 2-13

FOLKOW B M HALLBACK Y LUNDGREN and L WEISS Structurally based increase of flow resistance in spontaneously hypertensive rats Acta physiol scand 1970 79 373-378

FOLKOW B , M HALLBACK Y LUNDGREN and L WEISS Renal vascular resistance in spontaneously hypertensive rats Acta physiol scand 1971 83 96-105

FOLKOW B M HALLBACK Y LUNDGREN and L WEISS The effects of immunosympathectomy on blood pressure and vascular reactivity in normal and spontaneously hypertensive rats Acta physiol scand 1972 84 512-523

FOLKOW B M HALLBACK Y LUNDGREN L WEISS I ALBRECHT and S JULIUS Analysis of design and reactivity of series coupled vascular sections in spontaneously hypertensive rats (SHR) Acta physiol scand 1974 90 654-656

FOLKOW B P HEDNER B LISANDER and E RUBINSTEIN Release of cortisol upon stimulation of the hypothalamic defence area in cats Försvarsmedicin 1967 3 Suppl 2 114-119

FOLKOW B B LISANDER R S TUTTLE and S C WANG Changes in cardiac output upon stimulation of the hypothalamic defence area and the medullary depressor area in the cat Acta physiol scand 1968 72 220-233

FOLKOW B B LÖFVING and S MELLANDER Quantitative aspects of the sympathetic neuro-hormonal control of the heart rate Acta physiol scand 1956 37 363-369

FOLKOW B S MELLANDER and B ÖBERG The range of effect of the sympathetic vasodilator fibers with regard to consecutive sections of the muscle vessels Acta physiol scand 1961 53 7-22

FOLKOW B and E NEIL Circulation London-New York-Toronto Oxford Univ Press Inc 1971

FOLKOW B and B ÖBERG Autoregulation and basal tone in consecutive vascular sections of the skeletal muscles in reserpine-treated cats Acta physiol scand 1961 53 105-113

FOLKOW B and E H RUBINSTEIN Cardiovascular effects of acute and chronic stimulations of the hypothalamic defence area in the rat Acta physiol scand 1966 68 48 57

FRANK E Bestehen Beziehungen zwischen chromaffinem System und der chronischen Hypertonie des Menschen? Ein kritischer Beitrag zu der Lehre von der physio-pathologischen Bedeutung des Adrenalins Dtsch Arch klin Med 1911 103 397-412

FRIEDMAN S M C L FRIEDMAN and M NAKASHIMA Cationic shifts and blood pressure regulation Circulat Res 1957 5 261 267

FRIES E D Hemodynamics of hypertension Physiol Rev 1960 40 27 54

FROHLICH E D V J KOZUL R C TARAZI and H P DUSTAN Physiological comparison of labile and essential hypertension Circulat Res 1970 26 and 27 Suppl 1 55-69

FURUYAMA M Histometrical investigations of arteries in reference to arterial hypertension Tohoku J exp Med 1962 76 388-414

GRAFF CH R BAUMANN H ZIPRIAN W GÖDICKE und W HARTRODT Das Verhalten vegitativer und biochemischer Parameter bei essentiellen Hypertonikern während psychischer Stress-situationen Dtsch Gesundh Wes 1971 26 6-11

GRANT R P LINDGREN A ROSÉN and B UVNAS The release of catechols from the adrenal medulla on activation of the sympathetic vasodilator nerves to the skeletal muscles in the cat by hypothalamic stimulation Acta physiol scand 1958 43 135-154

GREENE M A R FRIEDLANDER A J BOLTAX C G HADJI GEORGE and G A LUSTIG Distensibility of arteries in human hypertension Proc Soc exp Biol Med 1966 121 580-585

GULL W W and H G SUTTON On the pathology of the morbid state commonly called chronic Bright s disease with contracted kidney (Arterio-capillary fibrosis) Trans Roy Med Chir Soc 1872 55 273 326

GUTMAN M C and H BENSON Interaction of environmental factors and systemic arterial blood pressure: A review Medicine 1971 50 543-553

GUYTON A C T G COLEMAN A W COWLEY K W SCHEEL R D MANNING and R A NORMAN Arterial pressure regulation Overriding dominance of the kidneys in long term regulation and in hypertension Amer J Med 1972 52 584-594

HALLBÁCK M Y LUNDGREN and L WEISS Reactivity to nor adrenaline of aortic strips and portal veins from spontaneously hypertensive and normal rats Acta physiol scand 1971 81 176-181

HENRY J P J P MEEHAN and P M STEPHENS The use of psychosocial stimuli to induce prolonged systolic hypertension in mice Psychosom Med 1967 29 408-432

HENRY J P and J C CASSEL Psychosocial factors in essential hypertension Amer J Epidem 1969 90 171 199

HERD J A W H MORSE R T KELLEHER and L G JONES Arterial hypertension in the squirrel monkey during behavioral experiments Amer J Physiol 1969 217 24-29

HILTON S M Inhibition of baroceptor reflexes on hypothalamic stimulation J Physiol (Lond) 1963 165 56-57

HORWITZ D B V CLINESCHMIDT J M VAN BUREN and A K OMMAYA Temporal arteries from hypertensive and normotensive man Reactivity to norepinephrine and characteristics of alpha-adrenergic receptors Circulat Res 1974 34 and 35 Suppl I 109-115

HUCHARD H Maladies du coeur et des vaisseaux Paris Doin 1889

HUTCHINS P M and A E DARNELL Observations of a decreased number of small arterioles in spontaneously hypertensive rats Circulat Res 1974 34 and 35 Suppl I 161-165

IRIUCHIJIMA J Cardiac output and total peripheral resistance in spontaneously hypertensive rats Jap Heart J 1973 14 267-272

ISAKSSON, O Influence of in vitro mechanical activity in protein synthesis and amino acid transport in perfused rat heart Göteborg Götatryckeriet 1972

JOHANSSON B B LJUNG T MALMFORS and L OLSON Prejunctional supersensitivity in the rat portal vein as related to its pattern of innervation Acta physiol scand 1970 Suppl 349 5–16

JOHANSSON B Determinants of vascular reactivity Fed Proc 1974 33 121-126

JOHNSON G On certain points in the anatomy and pathology of Bright s disease of the kidney II On the influence of the minute blood-vessels upon the circulation Trans Roy Med Chir Soc 1868 51 57-78

JONES A W Altered ion transport in vascular smooth muscle from spontaneously hypertensive rats Circulat Res 1973 33 563 572

JULIUS S and J CONWAY Hemodynamic studies in patients with borderline blood pressure elevation Circulation 1968 38 282-288

JULIUS S A V PASCUAL and R LONDON Role of parasympathetic inhibition in the hyperkinetic type of borderline hypertension Circulation 1971 44 413-418

JULIUS S A V PASCUAL K REILLY and R LONDON Abnormalities of plasma volume in borderline hypertension Arch Intern Med 1971 a 127 116-119

JULIUS S A V PASCUAL R SANNERSTEDT and C MITCHELL Relationship between cardiac output and peripheral resistance in borderline hypertension Circulation 1971 b 43 382-390

JULIUS S and M A SCHORK Borderline hypertension – a critical review J chron Dis 1971 23 723-754

KALIS B L R E HARRIS M SOKOLOW and L G CARPENTER Response to psychological stress in patients with essential hypertension Amer Heart J 1957 53 572 578

KERNOHAN J W E A ANDERSON and N M KEITH The arterioles in cases of hypertension Arch Intern Med 1929 44 395-423

KORNER P I M J WEST J SHAW and J B UTHER Steady-state ' properties of the baroreceptor-heart rate reflex in essential hypertension in man Clin Exp Pharm Physiol 1974 1 65-76

LANG G F Gipertonicheskaia Bolezn Moscow 1950 (cited from Simonson and Brožek 1959)

LIEBOW A A Situations which lead to changes in vascular patterns In Handbook of Physiology 1963 Circulation Vol 2 1251-1276

LISANDER B Factors influencing the autonomic component of the defence reaction Acta physiol scand 1970 Suppl 351

LOUIS, W J and A E DOYLE The use of a double-isotope derivative plasma assay for noradrenaline and adrenaline in the diagnosis and localization of phaeochromocytoma Austr N Z J Med 1971 3 212-217

LOUIS W J A E DOYLE S N ANAVEKAR C I JOHNSTON L B GEFFEN and R RUSH Plasma cathecholamine Dopamine beta-hydroxylase and renin levels in hypertension Circulat Res 1974 34 and 35 Suppl 1 57-61

LOUIS W J S SPECTOR R TABEI and A SJOERDSMA Synthesis and turnover of norepinephrine in the heart of the spontaneously hypertensive rat Circulat Res 1969 24 85-91

UND-JOHANSEN P Hemodynamics in early essential hypertension Acta med scand 1967 Suppl 482

LUNDGREN Y Adaptive changes of cardiovascular design in spontaneous and renal hypertension Acta physiol scand 1974 Suppl 408

LÖFVING B and S MELLANDER Some aspects of the basal tone of the blood vessels Acta physiol scand 1956 37 134-141

MAHOMED F A Chronic Bright s disease without albuminuria Guy s Hosp Rep 1881 25 295-416

McCUBBIN J W J H GREEN and J H PAGE Baroreceptor function in chronic renal hypertension Circulat Res 1956 4 205-210

MENDLOWITZ M Vascular reactivity in systemic arterial hypertension Amer Heart J 1973 85 252-259

MENDLOWITZ M N E NAFTCHI R L WOLF and S E GITLOW Vascular reactivity in the patient with essential hypertension and hypertension of renal origin Amer J Cardiol 1962 9 680–684

MIALL W E Heredity and hypertension Practitioner 1971 207 20–27

MORGAN, H E J R NEELY R E WOOL C LIÉBECQ H LIEBERMEISTER and C R PARK Factors affecting glucose transport in heart muscle and erythrocytes Fed Proc 1965 24 1040–1045

MORITZ A R and M R OLDT Arteriolar sclerosis in hypertensive and normotensive individuals Amer J Path 1937 13 679 728

NAGAOKA A K KIKUCHI and Y ARAMAKI Participation of tissue electrolytes and water to the spontaneous hypertension in rats Jap Circulat J 1970 34 489–497

NESTEL P J Blood-pressure and catecholamine excretion after mental stress in labile hypertension Lancet 1969 1 692–694

NORBERG K -A and B HAMBERGER The sympathetic adrenergic neuron Acta physiol scand 1964 63 Suppl 238

NOSAKA S and K OKAMOTO Modified characteristics of the aortic baroreceptor activities in the spontaneously hypertensive rat Jap Circulat J 1970 34 685–693

NOSAKA S and S C WANG Carotid sinus baroreceptor functions in the spontaneously hypertensive rat Amer J Physiol 1972 222 1079–1084

OKAMOTO K Spontaneous hypertension in rats Int Rev Exp Path 1969 7 227 270

OKAMOTO K Spontaneous hypertension Tokyo Igaku Shoin Ltd 1972

OKAMOTO K and K AOKI Development of a strain of spontaneously hypertensive rats Jap Circulat J 1963 27 282 293

PAGE I H and J W McCubbin The physiology of arterial hypertension In Handbook of Physiology 1965 Circulation Vol 3 2163 2208

PFEFFER M.A Comparison of hemodynamic characteristics of normotensive and spontaneously hypertensive rats (With particular reference to the adaptability of the heart to sustained hypertension) Dissertation University of Oklahoma Graduate College 1972

PFEFFER M A and E D FROHLICH Hemodynamic and myocardial function in young and old normotensive and spontaneously hypertensive rats Circulat Res 1973 32 and 33 Suppl 1 28 35

PFEFFER, M A E D FROHLICH J M PFEFFER and K WEISS Pathophysiological implications of the increased cardiac output of young spontaneously hypertensive rats Circulat Res 1974 34 and 35 Suppl 1 235-242

PICKERING G W The peripheral resistance in persistent arterial hypertension Clin Sci 1936 2 209-235

PICKERING G High blood pressure London J & A Churchill Ltd 1968

PRINZMETAL M and C WILSON The nature of the peripheral resistance of arterial hypertension with special reference to the vasomotor system J clin Invest 1936 15 63-83

RODBARD S Negative feedback mechanisms in the architecture and function of the connective and cardiovascular tissues Perspect Biol Med 1970 13 507-527

RUSH R.A P E THOMAS S H KINDLER and S UDENFRIEND Serum dopamine-β-hydroxylase Symp on the sequelae of hypertension 1974 Roch Inst of Mol Biol p 27

SANNERSTEDT R Hemodynamic response to exercise in patients with arterial hypertension Acta med scand 1966 Suppl 458

SANNERSTEDT R S JULIUS and J CONWAY Hemodynamic responses to tilt and beta-adrenergic blockade in young patients with borderline hypertension Circulation 1970 42 1057-1064

SARNOFF S J and J H MITCHELL The control of the function of the heart In Handbook of Physiology 1962 Circulation Vol 1 489-532

SEN S R C TARAZI P.A KHAIRALLAH and F M BUMPUS Cardiac hypertrophy in spontaneously hypertensive rats Circulat Res 1974 35 775-781

SHIBAYAMA F S MIZOGAMI and H SOKABE Cardiovascular reactivity in hypertensive rats Jap Heart J 1971 12 68-78

SHORT D Morphology of the intestinal arteriols in chronic human hypertension Brit Heart J 1966 28 184-192

SHORT D S and A D THOMSON The arteries of the small intestine in systemic hypertension J Path Bact 1959 78 321-334

SIMONSON E and J BROŽEK Russian research on arterial hypertension Ann Intern Med 1959 50 129-193

SIVERTSSON R The hemodynamic importance of structural vascular changes in essential hypertension Acta physiol scand 1970 Suppl 343

SIVERTSSON R R SANNERSTEDT and Y LUNDGREN Central and peripheral hemodynamics in latent hypertension Proc Belg Med Res Council 1975 To be published

SMIRK F H and W H HALL Inherited hypertension in rats Nature (Lond) 1958 182 727-728

SMOOKLER H H K H GOEBEL M J SIEGEL and D E CLARKE Hypertensive effects of prolonged auditory visual and motion stimulation Fed Proc 1973 32 2105-2110

SPECTOR S J H FLEISCH H M MALING and B B BRODIE Vascular smooth muscle reactivity in normotensive and hypertensive rats Science 1969 166 1300-1301

STARLING E H The linacre lecture of the law of the heart (Cambridge 1915) London Longmans Green & Co 1918

SUWA N and T TAKAHASHI Morphological and morphometrical analysis of circulation in hypertension and ischemic kidney München-Berlin-Wien Urban & Schwarzenberg 1971

TARAZI R C M M IBRAHIM H P DUSTAN and C M FERRARIO Cardiac factors in hypertension Circulat Res 1974 34 and 35 Suppl 1 213-221

TOBIAN L and J T BINION Tissue cations and water in arterial hypertension Circulation 1952 5 754-758

Van CITTERS R L Occlusion of lumina in small arterioles during vasoconstriction Circulat Res 1966 18 199-204

Von CITTERS R L B M WAGNER and R F RUSHMER Architecture of small arteries during vasoconstriction Circulat Res 1962 10 668 675

WAGNER, J J WACKERBAUER und H H HILGER Arterielles Blutdruck- und Hertzfrequenzverhalten bei Hypertonikern unter Änderung des transmuralen Druckes im Karotissinusbereich Z Kreisl Forsch 1968 57 701-712

WALLIN B G W DELIUS and K E HAGBARTH Comparison of sympathetic nerve activity in normotensive and hypertensive subjects Circulat Res 1973 33 9-21

WALSH J.A C HYMAN and R F MARONDE Venous distensibility in essential hypertension Cardiovasc Res 1969 3 338 349

WEISS L Aspects of the relation between functional and structural cardiovascular factors in primary hypertension Acta physiol scand 1974 Suppl 409

WERKÖ L and H LAGERLÖF Studies on the circulation in man IV Cardiac output and blood pressure in the right auricle right ventricle and pulmonary artery in patients with hypertensive cardiovascular disease Acta med scand 1949 133 427-436

WEZLER K und A BÖGER Die Dynamik des arteriellen Systems Der arterielle Blutdruck und seine Komponenten Ergebn Physiol 1939 41 292 606

WIDIMSKÝ J M H FEJFAROVÁ and Z FEJFAR Changes of cardiac output in hypertensive disease Cardiologia 1957 31 381-389

WOLF S and H G WOLFF A summary of experimental evidence relating life stress to the pathogenesis of essential hypertension in man In Hypertension Ed E T Bell Minneapolis Univ Minn Press 1951

WOOLF C M Principles of biometry New York Chicago-San Francisco D van Nostrand Co Inc 1968

YAMORI Y Contribution of cardiovascular factors to the development of hypertension in spontaneously hypertensive rats Jap Heart J 1974 15 194-196

YAMORI Y M MATSUMOTO H YAMABE and K OKAMOTO Augmentation of spontaneous hypertension by chronic stress in rats Jap Circulat J 1969 33 399-409

ZANCHETTI A G BACCELLI and G MANCIA Cardiovascular effects of emotional behaviour In cardiovascular regulation in health and disease Ed C Bartorelli and A Zanchetti Istituto Ricerche Cardiovascolari Milano 1971 pp 17-32

ZANCHETTI A and A STELLA Neural control of renin release Clin Sci Mol Med 1975 48 In press

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 425

[illegible] MECHANISMS

An experimental study in the cat with special reference to the fastigial nucleus

by

JAN MARTNER

Göteborg 1975

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 425

An experimental study in the cat with special reference to the fastigial nucleus

JAN MARTNER

Göteborg 1975

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 425

From the Department of Physiology
University of Göteborg, Sweden

CEREBELLAR INFLUENCES ON AUTONOMIC MECHANISMS

An experimental study in the cat with special reference to the fastigial nucleus

by

JAN MARTNER

Göteborg 1975

This summary is based on studies reported in the following papers:

I *Interaction between the fastigial pressor response and the defence reaction*
B Lisander and J Martner Acta physiol scand 1973 87 359-367

II *Influences on colonic and small intestinal motility by the cerebellar fastigial nucleus*
J Martner Acta physiol scand 1975 In press

III *Influences on the defecation and micturition reflexes by the cerebellar fastigial nucleus*
J Martner Acta physiol scand 1975 In press

IV Effects on gastric motility from the cerebellar fastigial nucleus
B Lisander and J Martner Acta physiol scand 1975 In press

V Integrated somatomotor cardiovascular and gastrointestinal adjustments induced from the cerebellar fastigial nucleus
B Lisander and J Martner Acta physiol scand 1975 In press

The papers are referred to in the text by their Roman numerals

CONTENTS

INTRODUCTION 5

CEREBELLAR ANATOMY AND FUNCTION 5

PREVIOUS STUDIES OF CEREBELLAR AUTONOMIC INFLUENCES 8

Cerebellar cardiovascular influences 8

Cerebellar influences on gastrointestinal function 10

Cerebellar effects on bladder function 11

Miscellaneous cerebellar effects on autonomic hormonal control mechanisms 11

AIM OF THE PRESENT STUDY 12

METHODOLOGICAL CONSIDERATIONS 14

Stimulations 14

Operative procedures and recordings 15
- The cardiovascular system 15
- The gastrointestinal tract 15
- The bladder 16

Analysis of mechanisms 16
- Efferent pathways 16
- Background reflex control 16

Histological methods 17

RESULTS AND COMMENTS 18

Influences on the cardiovascular defence reaction 18

Influences on intestinal motility 19
- Effects in acutely laparotomized cats 19
- Effects in cats with Thiry-Vella loops 22

Influences on defecation and micturition 22
- Defecation 22
- Micturition 23

Influe ces on gastric motor function 23

Behavioural and gastric responses in conscious cats 25

GENERAL DISCUSSION 27

Functional and structural considerations 28

Level of integration 29

Importance of background reflex activity 31

Parallels to somatomotor functions 32

SUMMARY AND CONCLUSIONS 33

ACKNOWLEDGEMENTS 35

REFERENCES 36

INTRODUCTION

Our knowledge of cerebellar physiology has in the last decade been greatly expanded Thus the interactions between the neurons constituting the cerebellar cortex have been revealed as have their afferent and efferent connections (for ref see Eccles Ito and Szentágothai 1967) which has meant a firmer ground for the development of theories concerning cerebellar function (e g Eccles 1969 Marr 1969 Ito 1970 Gilbert 1974) The experimental studies forming the basis of present concepts of cerebellar function have mainly considered the somatomotor mechanisms Moreover the most obvious disturbances observed upon cerebellar lesions whether experimentally induced or brought about by disease are those of the somatomotor control It is therefore understandable that cerebellar influences on autonomic mechanisms have generally come in the background and have sometimes even been more or less overlooked

However it has long been known that autonomic responses can be induced from the cerebellum and in recent years several studies have lent considerable support to the idea that the cerebellum is indeed of considerable importance also for the regulation of autonomic mechanisms as will be outlined below

CEREBELLAR ANATOMY AND FUNCTION

For a detailed description of cerebellar organization the reader is referred to monographs by Jansen and Brodal (1954) and by Eccles et al (1967) and here only some principles of particular relevance for the present study will be briefly outlined

On phylogenetic grounds the cerebellum was earlier usually divided into the archicerebellum paleocerebellum and neocerebellum However recent anatomical and functional findings better correspond to a sagittally oriented organization according to which the cortex consists of a medial zone the vermis bordered by the intermediate or paravermal zones which are laterally flanked by the hemispheres The great majority of the cerebellar autonomic effects observed have been elicited from the medial zone the vermal cortex and from its efferent projections as will be further outlined below

The neuronal organization of the cerebellar cortex is strikingly uniform independent of natomical localization or functional involvement The cortex can be divided into three layers where the Purkinje cells form the middle or ganglionic layer which separates the superficial or molecular layer from the deeper or granular one In the latter deep layer the small excitatory granule cells are found together with the inhibitory Golgi cells The superficial molecular layer contains the stellate and the basket cells which like the Golgi cells function as inhibitory interneurons

The afferent input to the cerebellar cortex is delivered via the climbing fibres and the mossy fibres These afferents convey an abundance of information to the cortical integrator at the same time as they via

axon collaterals exert an excitatory drive on the cerebellar nuclei (see below) This afferent information to the cerebellum is derived from a variety of sources including the motor cortex and various subcortical motor neuron pools bulbar and spinal reflex integration centres and virtually all types of peripheral sensory receptors somatic as well as visceral (cf Jansen and Brodal 1954 Dow and Moruzzi 1958 Newman and Paul 1966a b 1969 Evarts and Thach 1969 Oscarsson 1973)

The climbing fibres are axons from neurons in the inferior olive and exert a powerful excitatory influence directly on the Purkinje cells The mossy fibres having connections both from higher and lower CNS levels make excitatory contacts with the granule cells Each of these latter neurons excites via their axons the parallel fibres, around 45 Purkinje cells (Eccles 1973) The parallel fibres excite also the Golgi stellate and basket cells which are all inhibitory in nature The former type of interneurons exert an inhibitory action on the granule cells thus completing a negative feedback loop The stellate and basket cells exert their inhibitory action on the Purkinje cells and in such an arrangement as to focus the incoming excitatory discharge on a selected number of Purkinje cells As a result of a steady stream of afferent impulses there is also during resting conditions a continuous activity in the Purkinje cells of the cortex as well as in the cells of the cerebellar nuclei which are as mentioned excited by collaterals from the climbing and mossy fibres

The only output from the cerebellar cortex is mediated by the Purkinje cell axons which convey a uniformly inhibitory influence on the cerebellar nuclei and on some of the vestibular nuclei which functionally may be regarded as cerebellar Collaterals from these axons also make contact with the Golgi and the basket cells The Purkinje cells of the medial or vermal zone of the cortex project mainly on the fastigial nuclei and also on the vestibular nuclei while the paravermal cortex projects on the interpositu nuclei (in man corresponding to the emboliform and globose nuclei) and some vestibular nuclei Lastly the Purkinje cells of the lateral hemispheric (neocerebellar) zones project upon the laterally placed dentate nuclei us the efferent connections from the cerebellum to other parts of the ous system emanate from these different sets of cerebellar nuclei which in turn exposed to a cortical inhibition as outlined above

In this context the fastigial nuclei and their efferent pathways are of particular interest since the great majority of the cerebellar autonomic influences seems to be conveyed by these connections The fastigial axons pass via direct fastigiobulbar pathways or via the uncinate fasciculus to their main targets the pontine and medullary reticular formation and the vestibular nuclei In addition they also establish ascending connections to thalamic nuclei (Thomas et al 1956) and to structures in the limbic system (Heath and Harper 1974)

By such general arrangements and by receiving and modulating a wealth of information from various parts of the central nervous system and from peripheral receptors the mentioned efferent connections efficiently modify outgoing motor commands into well coordinated motility patterns Whether similar principles of cerebellar coordination are engaged also in autonomic control is still largely unknown but seems a priori likely The first step to reveal such mechanisms is to explore whether autonomic effects

can be induced by stimulations of cerebellar nuclei or/and the corresponding cortical sections From this type of experiments a considerable bulk of evidence is available as will be outlined below A further step would be to investigate in more detail the patterns of autonomically mediated responses thus induced and to identify the efferent links and effector systems involved The present studies have in general been organized along such principles and may help to elucidate the exact mode of influence exerted by the cerebellum on the visceromotor and hormonal control systems

PREVIOUS STUDIES OF CEREBELLAR AUTONOMIC INFLUENCES

Cerebellar cardiovascular influences

Pioneer investigations of cerebellar influences on vegetative functions were performed already in the 19th century Thus in 1871 Owsjannikow reported that partial or total cerebellectomy in cats and rabbits had no influence on the blood pressure while the following year Eckhard observed an increase in heart rate and blood pressure following stimulation of posterior vermal sections in dogs Considerably later Dresel and Lewy (1924) confirmed that vermal stimulation in dogs and rabbits led to a blood pressure increase and there were also other scattered observations at this time concerning cerebellar influences on cardiovascular mechanisms as surveyed by Wiggers (1943a) and Dow and Moruzzi (1958) Although certain dis crepancies in results were obtained in these early exploratory studies there were some general trends which can be summarized as follows: Electrical stimulation of the cerebellar cortex could modify heart rate and blood pressure in both directions whereas cerebellar lesions did not appreciably change resting blood pressure More precise interpretations of these early results can hardly be made mainly because stimulation techniques were at that time poorly developed with considerable risks for current spread

Well controlled investigations on cerebellar autonomic control es pecially concerning cerebellar influences on cardiovascular reflexes were performed by Moruzzi (1938 1940 1947 1950) Moruzzi found that stimulation of the anterior cerebellar lobe could markedly inhibit vasomotor reflexes while it hardly changed resting blood pressure Thus depressor responses elicited from afferent vagal fibres were inhibited by concomitant cerebellar stimulation This was the case also with pressor responses whether induced by afferent sensory nerve stimulations or by bilateral carotid occlusion This latter finding was confirmed by e g Wiggers (1943b) Hoffer (1965) and Hoffer Ratcheson and Snider (1966) Relevant in this context are also observations by Smith and Nathan (1966) showing that lation of the inferior olive which by way of the climbing fibres pro- ts on the cerebellum could inhibit the depressor component of the caro- id sinus reflex

Fitting into this pattern of cerebellar influences on vasomotor reflexes are reports by Reis and Cuénod (1965) that cerebellectomy enhanced the carotid baroreceptor reflexes in decerebrated cats Vasomotor reflexes elicited by peripheral nerve stimulations were also affected by cerebellectomy which may change pressor responses into depressor ones (Ramu and Bergmann 1967) On the other hand cerebellectomy was reported to influence resting regional blood flow distribution only slightly and signifi cant effects were confined to a reduction of blood flow to red skeletal muscle (Sheridan and Reis 1972) Also cerebellar stimulations both in the cortex (Hoffer 1965) and in the fastigial nucleus (Mitra and Snider 1972) have been found to directly affect muscle blood flow

As evident from the abovementioned studies the impact of cerebellar cortical stimulations on the cardiovascular system is quite variable Besides the importance of background vasomotor activity the influence of anaesthesia on the response pattern has also been emphazised Thus circulatory

responses following cerebellar cortical stimulation have been reported to be markedly depressed or even reversed following barbiturate administration (Hoffer 1965 Hoffer et al 1966 Rasheed Manchanda and Anand 1970) Also the stimulation frequency used is of importance for the cerebellar cardiovascular responses (Hoffer 1965) Perhaps species differences are to be considered as well inasmuch as rabbits seem to be more prone to display pressor responses while more variable blood pressure responses have been reported in cats

In the majority of the mentioned investigations the cardiovascular influences were induced from the cerebellar cortex, especially from the vermal part However in recent years the interest has been directed to the cerebellar nuclei where the fastigial nucleus is of particular interest since it receives most of its Purkinje cell axons from the vermal cortex Already in 1954 Zanchetti and Zoccolini observed that the fastigial nucleus was potent in producing pressor responses as part of a sham rage pattern in thalamic cats Later it was shown that a considerable well reproducible pressor response could be induced in intact cats from a small area confined to the rostral fastigial pole (Achari and Downman 1969 1970 Miura and Reis 1969 1970) This pressor response also present after intercollicular decerebration and characterized by widespread sympathetic ac tivatio (Achari and Downman 1970) as well as by an inhibition of vagal discharge to the heart (Lisander nd Martner 1971b Achari Al-Ubaidy and Downman 1973) seems to be in some way related to the baroreceptor reflexes Thus Miura and Reis (1971) have described a site the paramedian reticular nucleus where an interaction between projections from the fastigi l nucleus and the carotid sinus nerves seems to take place and also Nathan (1972) has presented evidence for fastigial projections on bulbar structures engaged in vasomotor control Further the cardiovascular response pattern elicited by fastigial stimulation was closely similar to that evoked by baroreceptor unloading (Lisander and Martner 1971b Doba and Reis 1972a) and fastigial stimulation inhibits reflexly induced bradycardia whether produced by drugs like noradrenaline or phenyl diguanide (Achari et al 1973), by afferent sinus or vagal nerve stimulation (Hockman Livingston and Talesnik 1970 Gurevitch and Vyshatina 1973) or by carotid sinus distension (Lisander and Martner 1971b) Even the release of ADH as elicited by baroreceptor unloading was suppressed by fastigial stimulation (Hata and Miura 1974) Concerning the possible functional significance of these cerebellar influences Doba and Reis (1972a 1972b 1974) have suggested that the fastigial nucleus might participate in orthostatic circulatory eflex adjustments since the cardiovascular responses to tilting were impaired following bilateral fastigial lesions in cats

With respect to cerebellar influences on autonomic mechanisms associated with particular behavioural adjustments Moruzzi observed already in 1947 that cerebellar stimulation in acute thalamic cats could suppress their spontaneously elicited sham rage responses i e interfere with the hypothalamically integrated defence reaction and its characteristic somatomotor and autonomic adjustments Such sham rage responses could also appear as rebound phenomena at the cessation of cerebellar cortical stimulation or be precipitated by fastigial stimulations (Zanchetti and Zoccolini 1954) Further evidence for a cerebellar hypothalamic interaction was presented

by Ban et al (1956) who reported that both hypothalamic pressor and depressor responses were abolished by concomitant stimulation of the anterior cerebellar lobe In line with these reports Lisander and Martner (1971a) found that cortical stimulation of the anterior cerebellar lobe suppressed the autonomic components of the hypothalamic defence reaction including the cholinergic muscle vasodilatation

These observations together with the findings by Zanchetti and Zoccolini that fastigial stimulation can precipitate sham rage raise the question in which way the fastigial projection influence the hypothalamic defence reaction directly or via descending relay stations Further stimulation of the fastigial nuclei and adjacent cerebellar structures e g the lingula has been found also to induce oral behaviour in awake cats (Koella 1955 Reis Doba and Nathan 1973 Berntson Potolicchio and Miller 1973) and rats (Ball Micco and Berntson 1974) suggesting that a variety of behavioural and autonomic patterns controlled by the limbic system and the hypothalamus might be modified by cerebellar mechanisms Therefore one of the purposes of the present study was to explore whether and in which ways the cerebellum may affect autonomic neuroeffector systems other than the cardiovascular apparatus such as the gastrointestinal tract and the bladder and whether these autonomic adjustments are closely associated with particular behavioural changes or/and with cardiovascular adjustments A preliminary report covering part of the present results has been published (Lisander and Martner 1974)

Cerebellar influences on gastrointestinal function

Most of the relatively few reports dealing with cerebellar gastrointestinal influences have been concerned with the effects of cerebellectomy where constipation seems to be the most common finding (for ref see Dow and Moruzzi 1958) According to Bard et al (1947) removal ,of certain cerebellar parts including the nodulus, eliminate motion sickness dogs thus affecting also the autonomic gastrointestinal expressions In (1938) noted that cerebellectomy temporarily modified duodenal lity into a pattern of alternating periods of inactivity and excitation and Wolfe (1969) reported that restricted cerebellar lesions may produce gastric ulcerations

Concerning effects of cerebellar stimulation Voronin and Zimkina (1938) observed longlasting inhibitions of small intestinal motility when the vermis and cerebellar hemispheres of cats were stimulated Ban et al (1956) reported that gastric motility can be inhibited from the anterior cerebellar lobe in rabbits while both increased and decreased duodenal (Beller and Talan 1971) and gastric motility (Manchanda Tandon and Aneja 1972) have been reported in cats following cortical and fastigial stimulations However no attempts appear to have been made to explore the pathways and peripheral mechanisms involved in the cerebellar modulations of gastrointestinal control

Cerebellar effects on bladder function

Several investigations have reported cerebellar influences on mic turition but with somewhat conflicting results particularly in earlier ablation experiments (see Dow and Moruzzi 1958) Stimulations of the cerebellar cortex can elicit both increases and decreases of bladder tone in intact cats (e g Whiteside and Guyton 1952 Emerson et al 1961 Rasheed et al 1970) as well as in decorticate animals (Bruhn et al 1961) Chambers (1947) reported micturition upon stimulation of the fastigial nucleus and adjacent cerebellar structures while fastigial stimulations could inhibit the micturition reflex according to Bradley and Teague (1969) Both increases and decreases of bladder tone as elicited from the fastigial nucleus were reported by Beller and Tolan (1971) and by Manchanda and Bhattarai (1972) The latter authors also noted that the responses although persisting were markedly changed after intercollicular brain stem section Again however there appears to exist no detailed study where the cerebellar effects have been related to the peripheral pathways and neuroeffector mechanisms engaged in bladder control

Miscellaneous cerebellar effects on autonomic hormonal control mechanisms

One of the oldest observations concerning cerebellar influences on autonomic and hormonal control systems were made by Claude Bernard in 1858 who reported hyperglycemia as a result of cerebellar lesion Since then many tudies with often contradictory results have been concerned with the role of the cerebellum in glucose regulation At least part of the background for these effects may be a cerebellar influence on the sympathetic control of catecholamine release from the adrenal glands (Wada Seo and Abe 1935) but also other types of mechanisms may be involved Thus Kaplan (1938) reported changes in the glucose tolerance test lasting for up to 3 months following cerebellar removal There are also scattered reports concerning cerebellar influences on such autonomically nd hormonally mediated mechanisms as those governing thermoregulation and sexual function (for ref see Wiggers 1943a Dow and Moruzzi 1958)

It is clear from this brief survey concerning the role of the cerebellum in the regulation of autonomic and hormonal control systems that very little is known about the precise mechanisms involved Thus most results hitherto are of an exploratory nature mostly indicating that a cerebellar influence appears to exist but only seldom offering any information about involved pathways reflex arcs and levels of central integration

AIM OF THE PRESENT STUDY

Although many investigations have thus demonstrated autonomic and even hormonal effects from the cerebellum very little is known about by which ways such influences are exerted In the present series of investigations an analysis has been performed concerning the cerebellar influence on a number of defined autonomic fun tions which have earlier been extensively studied in this laboratory concerning other aspects of their central and reflex control. The interest was concentrated on the cerebellar fastigial nucleus since most of the cerebellar autonomic influences seem to be conveyed via this nucleus and the cortical region the vermis which projects on its neurons

In the first paper (I) the interrelation between the fastigial nucleus and the hypothalamic defence area was investigated as an extension of an earlier study which showed that the hypothalamic defence reaction could be suppressed from vermal parts of the cerebellar anterior lobe (Lisander and Martner 1971a) Since the Purkinje cell axons from this cortical area exert an inhibitory influence on the fastigial neurons (see earlier sections) it was considered of interest to explore the fastigial influence on the defence reaction

In the subsequent paper (II) the question was raised whether and in which ways the fastigial nucleus besides the cardiovascular influences induced from the fastigial pressor area conveys effects also on other autonomically innervated organ systems The interest was here concentrated upon effects on intestinal motility as elicited by fastigial stimulations with an analysis of the autonomic pathways involved

Extended information concerning the organization of cerebellar autonomic control might emerge from investigations of cerebellar influences on such functions that involve both somatomotor and autonomic mechanisms For such reasons the defecation mechanism was chosen having the additional advantage that possible cerebellar influences on autonomic response patterns conveyed by the sacral parasympathetic outflow could be studied his is the case also for the micturition reflex and paper III was therefore voted to an analysis of the cerebellar influence on the defecation and micturition mechanisms

The extrinsic nervous control of gastric motility is particularly well developed involving besides adrenergic inhibitory and cholinergic excitatory mechanisms also the vagal relaxatory fibres Utilizing a so far unknown transmitter these latter fibres control the reservoir function of the stomach (Martinson 1965) The nervous control of the stomach therefore allows for highly differentiated response patterns via the mentioned three efferent autonomic links and may if modified from the fastigial nucleus offer interesting information about the organization of cerebellar autonomic influences (paper IV)

In any study of autonomic function control of the background reflex activity is a prerequisite for a detailed analysis of the mechanisms involved and this is certainly true also when superimposed cerebellar control mechanisms are investigated Abdominal surgery profoundly interferes with gastrointestinal background tone mainly by adding reflex inhibitory effects

induced by peritoneal irritation intestinal handling etc Since these inhibitory reflexes appear to be conveyed mainly via the adrenergic sympathetic supply to the gastrointestinal tract (see Furness and Costa 1974) the cerebellar influence on neurogenic intestinal control was explored both before and afte such reflex mechanisms were superimposed by inflicting abdominal trauma or irritation These aspects were explored both in paper IV and V

Lastly further indications of the functional importance of the fastigial nucleus concerning autonomic control might be obtained from studies where this cerebellar part is stimulated in unanaesthetized animals with chronic indwelling electrodes and such experiments are described in the last paper (V) This technique has also the advantage of avoiding anaesthesia which in itself may alter cerebellar responses In these cats it was possible to study behavioural responses to fastigial stimulation in relation to simultaneously induced cardiovascular and gastrointestinal adjustments

METHODOLOGICAL CONSIDERATIONS

For a detailed description of methods the different papers should be consulted and only a general survey will be given below The experiments were performed on altogether 150 cats In the acute experiments chloralose was used as anaesthetic Cerebellar autonomic influences were evaluated by recording autonomic effector responses e g heart rate blood pressure regional blood flow gastrointestinal and bladder motility etc This type of recording was judged to serve the present purpose better than electrophysiological recordings which may give results difficult to translate into functional patterns For example as will be illustrated below fastigial stimulation enhances the sympathetic excitatory discharge to blood vessels while it suppresses prevailing activity in the sympathetic fibres that inhibit intestinal motility Thus if cerebellar effects were analysed only by recording changes in spike activity in e g the splanchnic nerves it would be very difficult if not impossible to interpret the discharge pattern obtained in terms of neurogenic effects on the cardiovascular and gastrointestinal effectors The net effect on electrical activity as recorded in splanchnic nerve bundles may then even appear to be largely unchanged despite pronounced cerebellar influences on both gastrointestinal motility and blood flow since the mentioned decrease and increase respectively in adrenergic discharge mediating these effects might more or less cancel out It would further be impossible to differentiate between vasodilator and vasoconstrictor fibres but in terms of elicited effector responses they mean the very opposite to each other

Stimulations

For central stimulations both in the hypothalamus and cerebellum the Horsley-Clarke technique was utilized Stimulation was performed with monopolar steel electrodes using constant current usually between 0 05-0 2 The uninsulated tips to the electrodes were about 100 μm in diameter udies of central nervous mechanisms using topical electric stimulations danger of undue current spread is always at hand In the present study is problem had to be particularly carefully considered because of the close proximity between the fastigial nucleus and the brainstem However the following factors strongly indicate that the obtained responses to topical stimulations were true fastigial effects and not due to current spread to bulbar structures First the responsive area was quite small and minor shifts (0 5 mm) in electrode position even towards the brainstem lead to a disappearance of the stimulation effects Second the responses also disappeared if a small tissue lesion was applied around the electrode tip Third histological examination confirmed that the stimulation sites were located in or close to the fastigial nucleus (see below) Fourth threshold intensities were often quite low and values below 0 05 mA were sometimes sufficient to elicit responses

Operative procedures and recordings

In the cats used for acute experiments a tracheal cannula was inserted to secure free airways and also to allow for artificial respiration To eliminate catecholamine release from the adrenals these glands were ligated in cats subject to laparotomy and hydrocortisone substitution was then given

A The cardiovascular system Arterial catheters allowed for recordings of heart rate and blood pressure In the chronic awake animals such registrations could be obtained by means of an indwelling aortic catheter inserted via the right carotid artery Blood flow was recorded as the venous outflow from the organ under study The neurogenic control of muscle blood flow was sometimes estimated by using the cross circulation technique The isolated calf preparation with intact nervous connections was then perfused with blood from a donor cat thus allowing for flow recordings that were undisturbed by experimentally induced pressure variations in the recipient (paper I)

B The gastrointestinal tract Gastric motor responses were recorded as isotonic volume changes in a large waterfilled intragastric balloon This method reflects net changes in gastric volume rather than individual contraction waves and has the advantage of avoiding iterations in gastric pressure that might per se induce gastric motor reflexes (for details see Jansson 1969a) The intragastric balloon was inserted via the esophagus in the actue experiments (paper IV) and via an earlier made gastric fistula in the chronic cats (paper V) Both methods had the advantage of not requiring laparotomy

Intestinal motility was in most cases recorded as volume changes in isolated intestinal segments which via a wide tube were connected to a reservoir containing Tyrode solution By changing the level of the container the intraluminal pressure could be adjusted to induce a desirable level of intrinsic intestinal motor ctivity against whi h e g the adrenergic impact on motility could be tested Like the situation in the stomach this kind of ecording generally reflects overall changes in tone in the intestinal segme t and is therefore more suitable for the present purpose than identification and classification of indi idual contraction waves Further the danger of mistaking respiratory movements for intestinal peristalsis will be reduced

In addition the following precautions were undertaken to differentiate gastrointestinal motor action from somatomotor effects 1 Neuromuscular blocking agents were often used and rtificial respiration given 2 In the laparotomized cats direct inspections of the stomach and intestine confirmed that gastrointestinal motor activity corresponded to the recorded changes in motility 3 When intestinal motility was recorded the intestinal segment under study could be brought entirely outside the abdomen thus abolishing every possible somatomotor i fluence on the recordings

The above mentioned method of recording intestinal motility required laparotomy To avoid acute abdominal surgery which profoundly influences intestinal motility and reflexes a segment of the small intestine was iso-

lated in one group of cats at an operation 1 2 weeks prior to the acute experiment (paper V) The ends of this segment emerged through the abdominal wall to form a so-called Thiry Vella loop In which motility can be recorded without inflicting any acute trauma In all cats with Thiry-Vella loops (paper V) and in a few of the acute experiments (paper II) intestinal motility was recorded as pressure changes in intraluminal balloons

The somatomotor component of the defecation reflex was registered as pressure changes in an intraabdominally placed balloon thereby reflecting straining movements which could also be easily observed by direct inspection (paper III)

C The bladder Bladder motility was recorded as intravesical pressure changes by an urethral catheter inserted transabdominally (paper III) Via a side branch the bladder could be filled with warm saline at different pressures The pressure level was very important for the outcome of the responses

Analysis of mechanisms

A Efferent pathways The efferent mechanisms mediating the cerebellar effects were analyzed by a combined use of antiadrenergic or anticholinergic drugs and nerve sectioning or by reversible cold blockade of peripheral nerves The nerves to be cut or reversibly blocked in the course of the experiment were dissected free with great caution

B Background reflex control Since modulation of autonomic reflex activity seems to be one important mechanism for cerebellar autonomic interaction it was necessary to maintain proper control of the activity in reflexes impinging on the organ under study Increased activity in these autonomic reflex arcs could be induced by various means e g by electrical mechanical or chemical stimulation of the respective afferent fibres Hence the vago-vagal relaxatory reflex was elicited by graded afferent stimulation f one vagal nerve while the other nerve was left intact for conveying the rent discharge (cf Jansson 1969b) The defecation reflex was elicited ther by applying a stimulation of the cut pelvic nerves on one side, or by mechanical stimulation of the rectal mucosa (cf Hultén 1969) These methods of initiating defecation complemented each other Inasmuch as the colonic motor and blood flow adjustments induced by the adequate mechanical stimulus could also be evoked by afferent pelvic nerve stimulation revealing that these responses were not due simply to any intramural reflex or to mechanical artifact

The intestino-intestinal (gastric) inhibitory reflex which proved to be of particular relevance for cerebellar gastrointestinal control could be initiated by several different stimuli Firts laparotomy inevitably induced long lasting neurogenic inhibitions of gastrointestinal motility by reflexes which could be reinforced by afferent stimulation of mesenteric nerves or by distension of an intestinal segment In the intact animals these reflex inhibitions could be reversibly elicited by intraabdominal injection of small amounts of irritating fluid such as acids or alkali The cats with Thiry-

Vella loops (paper V) were particularly suitable for studying the intestino-intestinal (gastric) inhibitory reflex since graded intestinal distensions could here be performed without laparotomy

Histological methods

Identification of the electrode tracks and stimulation sites was performed by histological examination of the cerebellum In papers II V the stimulation sites giving rise to cerebellar autonomic responses were plotted on maps of the relevant cerebellar structures It must however be realized that it is not possible to localize the stimulated area with greater accuracy than about 1 mm Thus if data obtained from the hypothalamus (Wise 1972) can be applied also on the cerebellum the current spread with the present stimulation parameters might be up to 0 5 mm Another 0 5 mm error comes from the size of the electrolytic lesions made to indicate the stimulation sites

RESULTS AND COMMENTS

Influences on the cardiovascular defence reaction (paper I)

Stimulation of the hypothalamic defence area induces a widespread but differentiated sympathetic discharge to the circulatory system causing blood pressure rise tachycardia increased vascular resistance in most vascular beds except in skeletal muscle and an adrenal catecholamine release In skeletal muscle an increased blood flow is instead induced due to activation of cholinergic vasodilator fibres and to the vasodilator effect of blood borne adrenaline As a continuation of an earlier study showing that the cerebellar cortex can suppress the autonomic components of the defence reaction (Lisander and Martner 1971a) the fastigial influence on these cardiovascular adjustments was investigated and special attention was paid to the changes in muscle blood flow

Fastigial stimulation which in itself produces an adrenergically mediated pressor response and a decreased muscle blood flow was found to suppress the hypothalamically induced cholinergic vasodilatation in the skeletal muscles whereas the adrenergically mediated components of the defence reaction were potentiated The fastigial inhibition of the neurogenic muscle vasodilatation might a priori be due to either a central suppression of the vasodilator fibre discharge or to an increased constrictor fibre discharge which hemodynamically interferes with the vasodilator fibre influences The latter possibility is a realistic one since the effects of the vasodilator fibres may be nearly abolished by a concomitant vasoconstrictor fibre activity (Folkow Öberg and Rubinstein 1964) and such vasoconstrictor fibre activity is indeed induced by stimulation in the fastigial pressor area

Therefore special arrangements had to be undertaken to investigate whether the fastigial stimulation also caused a suppression of vasodilator fibre activity per se Blood flow was therefore recorded in a cross-circulated, adrenergically blocked calf muscle preparation (see Methods) which was erfused at constant pressure from a donor cat In this way the impact of cholinergic vasodilator fibres could be studied without any interference y the vasoconstrictor fibres Even then the muscle blood flow increase ring defence area stimulation could be suppressed by fastigial stimulation suggesting that the fastigial neurons interfere centrally with the activation of the sympathetic cholinergic vasodilator fibres However whether this interaction occurs at the hypothalamic level or at lower levels of the neur axis is unclear Fastigial stimulation alone did not influence blood flow in the adrenergically blocked muscle vascular bed indicating that the cholinergic vasodilator fibres are not activated by stimulation of the fastigial neurons

In some cats treated with the adrenergic blocker guanethidine and hence deprived of the vasoconstrictor fibre influence fastigial stimulation could potentiate a delayed muscle vasodilator response induced by defence area stimulation This potentiated dilator response characterized by long latency and duration was unaffected by atropine but blocked by β-blocking agents When taken together these observations indicate that the fastigial stimulation potentiated the adrenaline release from the adrenal medulla

known to be induced by defence area stimulation (cf Grant et al 1958) Guanethidine does not block the hormonal component of the sympatho-adrenal system but rather tends to potentiate the peripheral effects of adrenaline (Abercrombie and Davies 1963)

A fastigial facilitation also seems to take place regarding the adrenergic vasoconstrictor fibre discharge to the intestinal cutaneous and muscle vascular beds as elicited by defence area stimulations This was evident from experiments where the vasoconstrictor fibre influence could be recorded without interference from circulating catecholamines changes in perfusion pressure or from baroreceptor reflex mod lations Such arrangements are necessary prerequisites for detecting the slight adrenergic vasoconstrictor fibre activation to the muscle vascular bed which can be elicited from the defence area as long as the baroreceptor reflexes are hindered from changing this response into one of vasoconstrictor fibre inhibition (cf Lisander 1971)

In conclusion the hypothesis that the cerebellar cortex and nuclei influenced the defence reaction in mutually throughout opposite directions could not be verified insofar as the fastigial nucleus exerted a facilitatory influence on the adrenergically mediated links but suppressed the cholinergic vasodilator fibre influence

These findings appear to fit well with reports by Achari t al (1973) who observed a mutual fastigial hypothalamic facilitation concerning the adrenergic but not of the behavioural components of the defence reaction At first sight it is somewhat surprising that fastigi l stimulation may suppress part of the defence reaction i e the cholinergic vasodil tation since such stimulations can induce sham rage in decorticate cats (Zanchetti and Zoccolini 1954) However the situation is different in i tact anaesthetized cats compared to that i decorticate animals where sham rage can be triggered by very slight stimuli of unspecific trivi l nature or may even occur spontaneously (Bard 1928) These res lts will be discussed more in detail later on

I fluences on intestinal motility

Effects in acutely laparotomized cats (paper II) In this study it was explored whether intestinal motility is influenced by fastigi l stimulation besides the mentioned effects on the autonomic control of the cardiovascular system Recordings of motility were performed i the jejunum ileum and colon and fastigial stimulation was found to i fluence motility i all these intestinal parts though in a differentiated way Thus the jejunum responded with both excitatory and inhibitory patterns whereas the ileum uniformly responded with increased motor acti ity Also the colon displayed an excitatory pattern with a tendency for the proximal parts to be more responsive than distal segme ts

Analysis of the autonomic mechanisms which conveyed the i testinal responses was performed by the use of different blocking drugs in combination with se tioning or cold blockade of the peripheral autonomic nerve supply It was then found that interruption of the parasympathetic pathways the vagi for the small i testine nd for the colon in addition the pelvic

that there is little activity to be inhibited

Effects in cats with Thiry Vella loops (paper V) To further elucidate the fastigial influence on intestinal motility and especially its relation to the intestino-intestinal inhibitory reflex arrangements were made to record motility atraumatically (see Methods) thus avoiding the elicitation of inhibitory reflexes In such a situation it is likely that the effects of fastigial stimulation on intestinal motility are different As shown in Fig 1 panel A ileal motility is now depressed by fastigial stimulation which was never seen when motility was recorded in laparotomized cats (paper II) Panel B reflects the situation after vagotomy illustrating that the extrinsic parasympathetic pathways are not necessary for eliciting the ileal inhibitory responses Subsequent laparotomy depresses the spontaneous ileal motility and the same fastigial stimulation which before laparotomy induced motility inhibition (panel A) now enhances intestinal motility (panel C) Evidently the initial inhibitory effects by fastigial stimulation are *not conveyed* via the vagal parasympathetic supply which means that they must be due to an activation of the adrenergic inhibitory supply suppressing the prevailing activity in intramural cholinergic neurons However once a reflex activation of adrenergic inhibitory discharge is induced by abdominal trauma, *the same fastigial stimulation tends to suppress this prevailing activity* In other words it appears as if the fastigial neurons enhance the adrenergic fibre activity to the intestine if initially low or absent but suppress it when it is initially marked In contrast concerning the adrenergic fibre control of the cardiovascular system only excitatory influences from the fastigial nucleus have been observed

Influences on defecation and micturition (paper III)

Since the fastigial nucleus evidently is involved in adrenergic regulation of both cardiovascular (paper I) and intestinal functions (paper II) it as considered of interest to investigate if also parasympathetically conyed autonomic patterns are influenced The micturition and defecation lexes were chosen as examples of such autonomic mechanisms Furthermore the latter reflex also serves as an example of a pattern in which both autonomic and somatomotor components are closely interwoven and might for this reason be of particular interest for analyses of the mode of cerebellar modulation

Defecation Mechanical rectal stimulation or afferent pelvic nerve stimulation elicits a reflex increase in colonic blood flow and motility as well as straining movements (cf Hultén 1969) All these adjustments associated with defecation were found to be influenced by fastigial stimulation Thus both the colonic motor response and the increased blood flow were reduced or totally inhibited while the straining movements were always entirely abolished by a concomitant fastigial stimulation In no case were any of these components of the defecation reflex enhanced by fastigial stimulation The stimulation sites influencing the defecation reflex were located *in the rostral fastigial pole*

In all these experiments adrenergic blockade was used to eliminate the above described (paper II) fastigial modulation of colonic motility via the adrenergic fibre supply which might interfere with the recordings of parasympathetically mediated responses This procedure was also necessary for obtaining a full expression of the parasympathetic colonic motility and vascular effects which are known to be suppressed by a prevailing activity in the sympathetic fibre supply (e g Hultén 1969) The results indicate that the fastigial neurons can markedly suppress all the components of the defecation reflex but at which level of the neuraxis this influence is exerted is unclear It is certainly not at the receptor level, e g by some type of neurogenic suppression of intramural mechanoreceptor sensitivity since the fastigial suppression of the defecation reflex was the same whether the reflex was induced by adequate mechanical stimulation of the rectal wall or by afferent pelvic nerve stimulation Spinal as well as bulbar and suprabulbar centres seem to be involved in defecation (cf Koppanyi 1930 Hess and Brügger 1943 Hultén 1969) and the cerebellar i teraction might occur at any of these levels

Micturition Fastigial stimulation was found to cause either facilitation or inhibition of bladder tone The background bladder tone as well as the electrode position in the fastigial nucleus could both affect the direction of the response Thus a reversal from excitation to inhibition of bladder tone could be observed upon even slight changes in electrode position Further it was sometimes possible to induce both increased and decreased motility from the same stimulation site depending on the initi l state of the bladder Thus if bladder tone was initially high, suppression of this parasympatheti cally mediated tone was lik ly to ensue but the same fastigial stimulatio could induce bladder contraction if started during a period of bladder inactivity Such a dependence on background eflex activity concerning bladder responses has been reported also in experime ts where the cerebellar cortex was stimulated (Whiteside and Guyton 1952) The suppression f bladder tone as a result of fastigial stimulation was not due to any ympathetic activation since it was unaffected by complete blockade of adrenergic fibres Therefore the fastigial inhibitory influence on the micturition ref lex must be ascribed to a central suppression of parasympathetic activity as also suggested by Bradely and Teague (1969)

The fastigial structures ffecting the parasympathetic bladder control had a larger external than those from which pressor nd intestinal motility responses were elicited Further it was not possible to distinguish distinct areas from which excitatory and inhibitory bladder effects could be elicited. Not seldom rebound bladder excitations were observed upon cessation of fastigial stimulations and rebound effects have been observed also i connection with sham rage responses (Moruzzi 1947 Zanchetti and Zoccolini 1954)

I fluences on gastric motor function (paper IV)

This study was undertaken to investigate possible fastigial i fluences on the particularly complex autonomic control of the stomach Since this orga i supplied with two sets of vagal fibres the excitatory and relaxa-

tory ones and also with sympathetic inhibitory fibres It provides an interesting model for testing cerebellar influences on various types of autonomic control The stomach also offers the advantage that the motility control can be conveniently studied without laparotomy or other surgical interferences that tend to elicit sympathetically mediated inhibitory gastric reflexes Moreover background cholinergic activity in the stomach can be easily controlled by graded efferent vagal stimulations and also graded vago-vagal relaxatory reflexes can be elicited

The results of the present study indicate that the fastigial nucleus can influence gastric motility in a complex way by evoking both suppression and facilitation (Fig 1) If however the prevailing experimental conditions were under strict control and properly considered when evaluating the results It proved possible to analyse the main mechanisms involved Theoretically changes in gastric motor function might be induced by discharge variations in the inhibitory adrenergic or excitatory cholinergic nervous pathways as well as via altered activity in the vagal relaxatory fibres and also hormonal mechanisms might be involved All these possibilities were taken into account when the fastigial effects on the stomach were explored

In intact animals fastigial stimulation elicited excitatory as well as inhibitory responses sometimes combined to form biphasic patterns After guanethidine the inhibitory gastric responses were either completely eliminated or sometimes only reduced but then with a changed and considerably delayed response pattern These observations indicate that the effects were mediated by sympathetic nerves and also to some extent by adrenal catecholamine release Parasympathetic blockade also interfered with these responses (Fig 1 panel B) but as will be discussed below these observations do not necessarily imply that activity changes in such pathways are involved Excitatory gastric responses to fastigial stimulation appeared at least in part to be caused by activation of the vagal cholinergic pathways since the effects persisted after high spinal cord transection but were abolished by atropine These results illustrate how the fastigial neurons can also excite parasympathetic pathways In this case the vagal excitatory es to the stomach

The vagal relaxatory fibres whose transmitter mechanism is unknown reflexly activated by e g gastric distension and even the low pressures exerted by the present recording balloon is usually sufficient to elicit this reflex (cf Abrahamsson and Jansson 1973) Both afferent and efferent pathways of this reflex run in the vagal nerves and it was found that fastigial stimulation suppressed this vago-vagal reflex thus displaying another way of cerebellar modulation of gastric tone Moreover this interaction illustrates how the cerebellar control of autonomic mechanisms is not only confined to the adrenergic and cholinergic pathways

In laparotomized animals a different type of motility responses was obtained After acute abdominal surgery or intraperitoneal injection of irritating fluid procedures which elicit sympathetically mediated inhibitory reflexes to the stomach fastigial stimulation always resulted in excitatory gastric responses independent of the direction of preceding stimulatory effects (Fig 1 panels A and C) As seen from the figure the initially obtained gastric inhibitions were by laparotomy reversed into excitatory responses

which were conveyed by adrenergic fibres and in all likelihood caused by fastigial suppression of adrenergic inhibitory reflexes to the stomach These results therefore agree closely with those described above concerning intestinal responses in cats with Thiry-Vella loops where also reversed responses were obtained after laparotomy If in animals with a Thiry-Vella loop a distending pressure was applied to this loop gastric motility was depressed via the intestino-gastric inhibitory reflex This reflex inhibition of gastric motility could be suppressed by a concomitant fastigial stimulation even when such a stimulation per se induced a depression of gastric tone (paper V)

However a prevailing cholinergic nervous activity whether intrinsic or extrinsic is a prerequisite for demonstrating the adrenergic inhibitory influence on gastrointestinal motility This presumably reflects the fact that the influence of the sympathetic inhibitory fibres to the gastrointestinal tract is almost entirely confined to an action on the intramural cholinergic ganglia to judge from morphological (Norberg 1964) and functional studies (Kewenter 1965 Jansson and Martinsson 1966 Hultén 1969) Accordingly no effects whatsoever of adrenergic discharge can be induced if no cholinergic activity is present (cf Fig 1 panels A and B) Whenever the cholinergic pathways were interrupted it was therefore necessary to induce such activity either via stimulation of the extrinsic parasympathetic nerves or by facilitating intramural ganglionic activity e g by exposing the intestine to a suitable distendi g pressure

The fastigial interaction with sympathetically conveyed intestino-gastric inhibitory reflexes persisted following intercollicular decerebration; i e structures cranial to the midbrain are not essential for this type of cerebellar modulation (paper IV)

Evidently fastigial neurons can modulate the activity in all the three efferent sets of autonomic fibres that contribute to gastric motility control and these influences are furthermore exerted from the same restricted fastigial section i e the fastigial pressor area Analysis of the frequency-esponse relationships for the fastigial effects revealed that those representing the cardiovascular and the gastric effects showed closely similar characteristics Thus there may be close anatomical and functional relationships between the fastigial neuron pools that influence these types of autonomic mechanisms; possibly even the same fastigi l neurons might be involved

To summarize the fastigial nucleus can induce inhibition of gastric motility by inducing an increased adrenergic discharge and/or an adrenal catecholamine release Increased gastric motility can be elicited by facilitation of vagal cholinergi discharge by inhibition of a prevailing activity in vagal r laxatory fibres and i situations f abdominal irritation or lesions, by suppression of adrenergic inhibitory reflexes Which of these mechanisms that dominates the pattern of the response in a given situation depends largely upon the prevailing background of autonomic activity

Behavioural and gastric responses in conscious cats (paper V)

To gain further information concerning the functional importance of the fastigial nucleus in autonomic control observations were made also in

unanaesthetized cats with indwelling fastigial electrodes In these cats arrangements were made for registration of gastric motility and blood pressure without any acute surgical intervention thus allowing for simultaneous observations of circulatory gastric and behavioural adjustments in response to fastigial stimulation The idea was to explore whether any particular behavioural patterns were associated with the autonomic responses or might be secondarily induced as a result of such autonomic adjustments

As in anaesthetized cats fastigial stimulation induced in the conscious freely moving animals pressor responses but in addition patterns of more or less obvious oral behaviours in this respect confirming findings by Reis et al 1973 and Berntson et al 1973 The character of these behavioural responses was partly dependent on the stimulation intensity Thus low current levels initiated sporadic licking of mouth and fur an activity which became more vigorous with increasing intensity At still higher intensities the grooming was replaced by biting and chewing and in one cat eating was induced All these responses started and ended within few seconds after onset and cessation of stimulation and could be easily reproduced The responses were furthermore performed in a well coordinated and stereotyped fashion and the animals showed no signs indicating that the stimulations might have been disagreeable These specific somatomotor responses did not take the form of individual limb movements or other types of restricted motility patterns which often seems to be the case when other cerebellar nuclei are topically stimulated in conscious animals (e g Chambers 1947 Koella 1955)

In contrast to the pronounced effects on blood pressure and behaviour fastigial stimulation did not conspicuously influence gastric motor activity and only in a few cases were gastric inhibitions observed resembling the adrenergically mediated relaxations in anaesthetized cats (paper IV) Neither was the fastigially elicited oral behaviour associated with any measurable increase in gastric hydrochloric acid secretion The absence of regularly appearing gastric responses in association with the oral behaviours indicates that the latter can hardly be secondary to fastigially induced gastric ad-

In subsequent acute experiments during anaesthesia on the same ani
fastigial stimulation elicited the earlier described inhibitory or bi
gastric responses After abdominal surgery these responses changed into uniformly excitatory effects indicating suppression of intestino-gastric inhibitory reflexes This gastric response pattern was thus similar to that found in the anaesthetized cats reported in paper IV Furthermore histological examination showed that it was elicited from the same cerebellar area i e from the rostral fastigial pole As a matter of fact most autonomic influences (paper I V) and also the oral behaviours reported in paper V were elicited from this area Thus the fastigial pressor area seems to form a site where many not necessarily interrelated kinds of autonomic and behavioural mechanisms may be modulated; observations which are in consonance with reports by Reis et al (1973)

GENERAL DISCUSSION

In recent years a considerable amount of information has accumulated confirming early observations indicating an involvement of the cerebellum also in functions beside its wellknown control of somatomotor mechanisms For example cerebellar influences on autonomic regulation especially concerning cardiovascular mechanisms have been repeatedly demonstrated Further there are reports of behavioural and emotional effects following cerebellar lesions (Peters and Mo jan 1971) and stimulations (Reis et al 1973 Bernison et al 1973 Ball et al 1974) The fastigial nucleus is of particular interest in this context since the great majority of the cerebellar autonomic adjustments reported in the literature have been ascribed to structures having their efferent pathways via this nucleus

From the rostral fastigial pole powerful and well reproducible pressor responses can be elicited by electrical stimulation (Achari and Downman 1969 1970 Miura and Reis 1969 1970) The present results indicate that this pressor area in addition to its cardiovascular influences is involved also in several other aspects of autonomic regulation For example colonic and small intestinal motility (paper II) defecation and micturition (paper III) and different reflex arcs involved in gastric motor control (paper IV) can be markedly influenced by fastigial stimulation Thus both the para sympathetic pathways vagal and sacral and the sympathetic inhibitory nerve supply can be modulated as well as the hormonal link of the sympatho-adrenal system This latter example of hormonal involvement is in agreement with recent demonstrations that fastigial stimulation can affect ADH secretion as well (Hata and Miura 1974) In addition oral behaviours can be elicited from the fastigial pressor area (paper V) confirming findings by Reis et al (1973)

It therefore appears as if a great variety of autonomic adjustments can be induced from a quite restricted section of the cerebellar efferent outflow involving virtually all links of the parasympathetic and sympatho-adrenal systems and consequently their subordinated effector organs However these different autonomic effects which can almost ll be induced from the same electrode position in the fastigial nucleus do not form the same type of regular and wellintegrated response patterns as those which can be elicited from e g the hypothalamic feeding area or defence area While these latter responses are always the same in direction it seems as if fastigial neurons exert rather a modulating influence on autonomic control mechanisms in general often damping prevailing activity when this is high but exerting a facilitating i fluence whenactivity is initially low Such a type of influence largely seems to hold for the gastrointestinal system while the fastigial influence on sympathetic activity to the cardiovascular system appears to be mainly of a facilitatory nature This is so even when the cardiovascular sympathetic discharge is enhanced by baroreceptor unloading (Achari and Downman 1970 Miura and Reis 1971 Lisander and Martner 1971b) or defence area stimulation (Achari et l 1973 paper I) This concentration of a variety of vegetative and hormonal influences to a very restricted part of the fastigi l nucleus is in line with observations that the fastigial neurons seem to be aggregated in colonies of related performance (Eccles et al 1972)

due to a descending direct interaction with neurons or receptors outside the central nervous system even though the principle of e g efferent neurogenic modulation of receptor sensitivity is generally accepted Such a view is furthermore supported by the following observations: First fastigial stimulation was found to modulate the defecation reflex equally efficiently whether the reflex was induced by a physiological excitation of rectal receptors or by afferent pelvic nerve stimulation (paper III) Second fastigial stimulation was found to influence all autonomic reflexes tested in this series of investigations with one notable exception Mechanical stimulation of the intestinal mucosa elicits an intestinal vasodilatation which is fairly unique by being conveyed via strictly intramural reflex connections (see Biber 1973) and this type of extraspinal autonomic reflex was not influenced by fastigial stimulation (Martner unpublished observation)

Cerebellar influences on spinal or/and bulbar autonomic structures appear as probable levels for interaction In fact several autonomic reflexes known to be relayed on these levels are liable to cerebellar modulation For example the cerebellum can influence cardiovascular (Moruzzi 1938 1940 Stella and Stevan 1962) micturition (Bradley and Teague 1969) and gastric reflexes (paper IV) Further these mentioned modulations are all present in animals subject to decerebration at pre or intercollicular levels showing that these particular cerebellar influences are not relayed at CNS centres cranial to mesencephalon Also the fastigial pressor response does not require higher levels of the nervous system since decerebration does not abolish the blood pressure increase upon fastigial stimulation (Achari and Downman 1969 Miura and Reis 1969)

There are however indications that also higher autonomic centres like the hypothalamus and the limbic system may be exposed to cerebellar influences concerning the vegetative functions that are integrated at these levels Thus the cerebellum can influence ADH release (Hata and Miura 1974) and also induce sham rage (Zanchetti and Zoccolini 1954) Further cardiovascular and gastric responses elicited from the hypothalamus can be influenced by cerebellar stimulation (Ban et al 1956) as is the case with autonomic components of the hypothalamic defence reaction (Lisander Martner 1971a paper I) However an interaction with relay centres for the ascending or descending pathways can of course not be excluded in these cases Thus the fibre system that controls the sympathetic cholinergic vasodilator fibres has synapses both in the mesencephalon and in the spinal cord (Lindgren 1955) Further indications of a cerebellar hypothalamic interrelation come from observations that some cardiovascular responses elicited by cerebellar stimulation are abolished by precollicular decerebration (Ban et al 1956) or by diencephalic coagulation (Ban Hilliard and Sawyer 1960 Sawyer Hilliard and Ban 1961)

Even if the results from the studies mentioned above clearly demonstrate an interaction between limbic hypothalamic influences and cerebellar influences they cannot be taken as conclusive evidence for the assumption that some cerebellar effects are really relayed at hypothalamic and limbic levels The mentioned interaction may well take place in centres at e g bulbar levels whose functions are affected by and dependent on both types of influences

There is however neuro-anatomical evidence for connections between

the cerebellum and the hypothalamus or/and the limbic system Such connections have been demonstrated both with electro-physiological methods (e g Ban and Inoue 1956 Anand et al 1959 Heath and Harper 1974) and with histological studies (Heath and Harper 1974)

Exactly how the fastigial induction of the oral behaviour is effectuated and which nervous structures that are involved is of considerable interest but is so far not known In case the cerebellum is capable of learning and memorizing patterns of somatomotor performance as proposed e g by Marr (1969) and Gilbert (1974) fastigial stimulation might have activated learned sequences of movements involved in oral behaviour It is however difficult to understand how such an admittedly crude interference with the delicate cerebellar machinery as a topical electrical stimulation implies can induce such complex and well coordinated motor performances A more reasonable explanation seems to be that the fastigial neurons exert a general facilitating influence on centres that normally initiate and integrate oral behavioural patterns e g the hypothalamic feeding area the limbic system etc The amygdala might here be of particular interest since oral behaviour can be particularly easily induced by stimulation in this region (cf Gloor 1960) and it was recently shown that the amygdala receive ascending projections from the fastigial nucleus (Heath and Harper 1974) Also a fastigial facilitation of bulbar relay centres might be of relevance for the oral responses since even rather complex motor performances can be integrated at these lower levels of the neuraxis (cf Bard and Macht 1958) The incitement of oral behaviour by unpleasant sensations induced by cerebellar stimulation (Chambers 1947 Koella 1955) is a third possibility which however appears nlikely since self-stimulation can be obtained from the same areas that produce these response patterns (Ball et al 1974)

Importance of background reflex activity

As mentioned bove it is most likely that the cerebellum exerts its autonomic influence by modifying reflex relay stations and higher centres controlling utonomic neuroeffectors If so the prevailing reflex activity must be of tmost importance for determining the response to cerebellar stimulatio which also ppears to be the case Thus cardiovascular (Moruzzi 1938 1940) gastrointestinal (Manchanda et al 1972 paper II IV and V) and bladder responses (Whiteside and Guyton 1952 paper III) are all very much dependent on the initial level of autonomic acti ity and impinging r flex influences For example quite different gastrointestinal responses occur upon fastigi l stimulation depending on whether intestino-intestinal (gastric) inhibitory reflexes are operating or not When such reflexes are active they are subject to fastigial suppression leading to motility increases (paper II IV V) If however no such spinal sympatheti reflex activity is going on the same fastigial stimulation may now depress motility by nhancing the inhibitory adrenergic discharge (Fig 1) Hence when the cerebellar influence on autonomic function is studied it i of utmost importance to have full control of and insight into the level of activity in both the various fferent autonomical pathways and in the subordi

nated effector systems Otherwise the results are likely to be very confusing apparently lacking all regularity and order

Parallels to somatomotor functions

Although the present knowledge about cerebellar autonomic control is only fragmentary as outlined above it seems reasonable to assume that it operates according to similar though not necessarily identical principles as those utilized in the somatomotor coordination The unique and very uniform neuronal architecture throughout the cerebellum speaks in favour of such a view However there are certainly several points where the organization of autonomic and somatomotor efferent control systems differ even if these two systems no doubt show many close similarities

To effectuate coordination of somatomotor movements the cerebellum receives information from a variety of somatic sensory receptors and from higher centres as well Also afferents from visceral organs have been found to project on the cerebellum (Widén 1955 Newman and Paul 1966a 1969 Langhof Höppener and Rubia 1973) converging on the same population of cerebellar neurons as the somatic afferents (Bremer and Bonnet 1951 Newman and Paul 1966b) Moreover the background activity in somatomotor reflex arcs can modify cerebellar somatomotor influences (Moruzzi 1936) as is also the case with background reflex activity in autonomic responses to cerebellar stimulation Rebound phenomena well known from experiments on cerebellar somatomotor control are seen also in association with autonomic influences e g on the bladder (paper III) In addition one and the same cerebellar stimulation may influence both the somatomotor and autonomic components of combined patterns like those seen in sham rage (Zanchetti and Zoccolini 1954) defecation (paper III) or the oral behaviour which was predominantly associated with cardiovascular adjustments (paper V) Many situations like physical activity emotional behavioural patterns tc require concomitant integrations of complex somatomotor and autono- events which must be well synchronized and timed to occur in proper quences Presumably the cerebellum participates in such complex coordinations and the present results lend support to such a view illustrating some of the principles for the cerebellar modulation combined of autonomic and somatomotor adjustments

Thus there is much to indicate that the cerebellum is involved in autonomic regulation and further that such control is carried out according to similar principles as those involved in the somatomotor coordination though presumably with adaptations according to the somewhat different functional and anatomical characteristics of the two systems

SUMMARY AND CONCLUSIONS

The present study was carried out to investigate cerebellar control of autonomic functions. For this purpose stimulation of the cerebellar fastigial nucleus seemed to be a suitable approach since the cerebellar influence on bulbospinal mechanisms is conveyed via the axonal connections of the nuclear neurons. This cerebellar nucleus receiving its cortical connections predominantly from the medially located vermis region contains in its rostral pole a restricted area which upon stimulation produces pressor responses and from which also a number of other autonomic influences and behavioural changes could be induced as outlined below:

1 Fastigial stimulation was found to suppress the cholinergic vasodilatation in skeletal muscle elicited from the hypothalamic defence area. This suppression was due to a fastigial modulation of activity in the central pathways mediating the cholinergic vasodilatation but whether the effect was exerted at the hypothalamic mesencephalic medullary or spinal relay stations is not clear. The adrenergic links of the defence reaction were on the other hand potentiated by fastigial stimulation. Hence the fastigial impact on the defence reaction was a differentiated one implying both facilitation and inhibition. Some functional implications of this fastigial interaction with the defence reaction were discussed.

2 Intestinal motility was influenced from the fastigial nucleus but differently so in laparotomized cats compared with cats not subjected to any acute abdominal surgery or irritation. Thus in laparotomized cats both excitatory and inhibitory jejunal responses were recorded while the ileum and colon uniformly responded with contraction. Abdominal surgery reflexly induces inhibitory adrenergic discharge to the gastrointestinal tract. The contractions recorded in the ileum and the colon following fastigial stimulation were in all likelihood due to a fastigial suppression of such prevailing inhibitory adrenergic discharge. A similar fastigial suppression of sympathetic gastrointestinal discharge was obtained when intestino-intestinal inhibitory reflexes were induced by intestinal distension or by afferent mesenteric nerve stimulation.

In contrast when intestinal motility was recorded atraumatically fastigial stimulation induced inhibition of ileal motility then reflecting a fastigial facilitation of sympathetic activity to the intestine. If however in these cats a high background sympathetic gastrointestinal discharge was initiated e.g. by abdominal trauma then fastigial stimulation instead suppressed this sympathetic discharge hereby changing the ileal motility response pattern into one of excitation.

3 Fastigial stimulation was potent in influencing both somatomotor and autonomic reflex adjustments involved in defecation. Thus the straining movements as well as the parasympathetically conveyed increases in colonic blood flow and motility were found to be strongly suppressed by concomitant fastigial stimulation. The micturition reflex as well was influenced from the fastigial nucleus but the direction of the responses depended

on the stimulation site in the fastigial nucleus Further these responses to fastigial stimulation were also influenced by background bladder tone suppression occurring when the parasympathetic reflex activity was initially high and vice versa They were predominantly due to a fastigial modulation of parasympathetic discharge to the bladder

4 Gastric motility was affected from the fastigial nucleus in various ways depending in each case on prevailing activity in the three efferent autonomic links which affect gastric motility Inhibitory responses could be initiated by a fastigial increase in adrenergic fibre discharge or/and in adrenal catecholamine release Excitatory responses could be induced by an increase in vagal cholinergic fibre discharge but also by a fastigial inhibition of a prevailing reflex activity in the vagal relaxatory fibres Further fastigial stimulation could induce gastric excitatory responses also by suppression of a prevailing sympathetic inhibitory fibre discharge as in the intestine whenever such activity was present as induced by e g abdominal surgery or intestinal distension

5 In conscious cats fastigial stimulation evoked a well coordinated oral behaviour such as licking grooming biting and chewing; sometimes even eating As in anaesthetized cats this topical fastigial stimulation induced pressor responses as well but the oral behaviour was not accompanied by any conspicuous adjustments of gastric function in terms of either motility or acid secretion When the same stimulations were repeated during chloralose anaesthesia in these chronic animals gastric responses similar to those described above for anaesthetized cats appeared Histological examination revealed that the responsive cerebellar area i e the rostral fastigial pole was the same for the behavioural changes as for most of the autonomic responses reported in the present investigation

It is thus possible to induce a variety of autonomic effects from a restricted fastigial area involving virtually all links of the parasympathetic system and the sympathetic system including the adrenals The results therefore indicate that the cerebellum is involved in the regulation of a variety of autonomic functions perhaps according to principles, similar to those valid for cerebellar somatomotor control The cerebellar influence is not only confined to such selected autonomic responses closely associated with e g particular somatomotor adjustments such as the defence reaction the feeding response the act of defecation etc but appears to affect virtually all types of autonomic mechanisms including reflexes engaged in bulbospinal homoeostatic mechanisms and resulting in cardiovascular gastrointestinal or urinary bladder adjustments Probably the cerebellar influence takes the form of a modulating and fine-adjusting influence which is exerted mainly at spinal and bulbar levels but it appears likely that cerebellar autonomic interactions may occur also at higher levels by means of e g fastigial projections on hypothalamic and limbic structures

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my teacher in research docent Björn Lisander for his guidance support and co-operation I am also deeply grateful to professor Björn Folkow for invaluable help and advice which have been of great importance for this work

Thanks are due to my colleagues on the department of physiology for valuable discussions and comments For constructive criticism I am obliged to docent Hans Hultborn I wish to thank Miss Birgitta Hillvärn and Mrs Kerstin Bongsell for excellent technical assistance For skilful typing of the manuscripts I want to thank Miss Lena Nimmerfors and Mrs Kerstin Lundgren The help from civ ing Lars Stage animal keeper Gösta Magnusson and photographer Thomas Palm is gratefully acknowledged

This research has been sponsored by grants from the Swedish Medical Research Council (No B74-14X 16-10C) from Svenska Sällskapet för Medicinsk Forskning and from the Faculty of Medicine University of Göteborg

ABERCROMBIE G F and B N DAVIES The action of guanethidine with particular reference to the sympathetic nervous system Brit J Pharmacol 1963 20 171 177

ABRAHAMSSON H and G JANSSON Vago-vagal gastro-gastric relaxation in the cat Acta physiol scand 1973 88 289 295

ACHARI N K S AL-UBAIDY and C B B DOWNMAN Cardiovascular responses elicited by fastigial and hypothalamic stimulation in conscious cats Brain Res 1973 60 439-447

ACHARI N K and C B B DOWNMAN Autonomic responses evoked by stimulation of fastigial nuclei in the anaesthetized cat J Physiol (Lond) 1969 204 130P

ACHARI N K and C B B DOWNMAN Autonomic effector responses to stimulation of nucleus fastigius J Physiol (Lond) 1970 210 637 650

ANAND B K C L MALHOTRA B SINGH and S DUA Cerebellar projections to limbic system J Neurophysiol 1959 22 451-457

BALL G G D J MICCO Jr and G G BERNTSON Cerebellar stimulation in the rat: Complex stimulation-bound oral behaviors and self stimulation Physiol Behav 1974 13 123-127

BAN T J HILLIARD and C H SAWYER Autonomic and electroencephalografic responses to stimulation of the rabbit cerebellum Anat rec 1960 136 309

BAN T and K INOUE Interrelation between anterior lobe of cerebellum and hypothalamus II Cerebellar evoked potential Med J Osaka Univ 1956 7 841 857

N T K INOUE S OZAKI and T KUROTSU Interrelation between anterior lobe of cerebellum and hypothalamus in rabbit Med J Osaka Univ 1956 7 101 115

P A diencephalic mechanism for the expression of rage with special reference to the sympathetic nervous system Amer J Physiol 1928 84 490-515

BARD P and M B MACHT The behaviour of chronically decerebrate cats In Ciba Foundation symposium on the neurological basis of behaviour Ed G F W Wolstenholme and C M O'Connor J & A Churchill Ltd London 1958

BARD P C N WOOLSEY R S SNIDER V B MOUNTCASTLE and R B BROMILEY Delimitation of central nervous mechanisms involved in motion sickness Fed Proc 1947 6 72

BELLER N N and M I TALAN Significance of the cerebellar nuclei for vegetative responses to the stimulation of the afferent visceral areas of the cat cerebellar cortex Sechenov Physiol J USSR 1971 LVII 29-37

BERNARD C Leçon sur la physiologie et la pathologie du system nerveux Vol 1 J B Baillière et Fils Paris 1858

BERNTSON G G S J POTOLICCHIO Jr and N E MILLER Evidence for higher functions of the cerebellum: Eating and grooming elicited by cerebellar stimulation in cats Proc Nat Acad Sci Wash 1973 70 2497 2499

BIBER B Vasodil tor mechanisms in the small intestine An experimental study in the cat Acta physiol scand Suppl 401 1973

BLOOMFIELD S and D MARR How the cerebellum may be used Nature 1970 227 1224-1228

BRADLEY W E and C T TEAGUE Cerebellar influence on the micturition reflex Exp Neurol 1969 23 399-411

BREMER F et V BONNET Convergence et interaction des influx affére ts dans l écorce cérébelleuse principe fonctionnel du cervelet J Physiol (Paris) 1951 43 665-667

BRUHN J M J O FOLEY G M EMERSON and J D EMERSON A tonomic response to cerebellar stimulation in the decorticate cat Amer J Physiol 1961 201 700-702

CHAMBERS W W Electrical stimulation of the i terior of the cerebellum in the cat Amer J Anat 1947 80 55-93

DOBA N and D J REIS Changes in regional blood flow and cardiodynamics evoked by electrical stimulation of the fastigial nucleus in the cat and their similarity to orthostatic reflexes J Physiol (Lond) 1972 a 227 729 747

DOBA N and D J REIS Cerebellum: role in reflex cardiovascular adjustment to posture Brain Res 1972 b 39 495-500

DOBA N and D J REIS Role of the cerebellum nd the vestibular pparatus in regul tion of orthostatic reflexes in the cat Ci culat Res 1974 34 9-18

DOW R S and G MORUZZI The physiology and pathology of the cerebellum Univ of Minnesota Press Minneapolis 1958

DRESEL K und F H LEWY Die Lokalisation vegetativer Zentren im Kleinhirn Deutsche Z itschrift für Nervenheilkunde 1924 81 82-83

ECCLES J C The dynamic loop hypothesis of movement control In I formation processing in the nervous system Ed K N Leibovic Springer Verlag New York Heidelberg Berlin 1969

ECCLES J C The cerebellum as a computer: patterns i space and time J Physiol (Lond) 1973 229 1 32

ECCLES J C M ITO and J SZENTAGOTHAI The cerebellum as a neuronal machine Springer Verlag Berlin Heidelberg New York 1967

ECCLES J C N H SABAH R F SCHMIDT and H TABORIKOVA Mode of operation of the cerebellum in the dynamic loop control of movement Brai Res 1972 40 73-80

ECKHARD C Beiträge Anat und Physiol (Giessen) 1872 6 IV Abhandl 51-94 VII Abhandl 177-192 (cited by Wiggers 1943 a)

ELLISON G D and A ZANCHETTI Specific appearance of sympathetic cholinergic vasodilatation in muscles during conditioned movements Nature 1971 232 124 125

EMERSON J D J M BRUHN G M EMERSON and J O FOLEY Autonomic response to chronic electrode stimulation of cerebellum Amer J Physiol 1961 201 697 699

EVARTS E V and W T THACH Motor mechanisms of the CNS: Cerebrocerebellar interrelations Annu Rev Physiol 1969 31 451 498

FOLKOW B B ÖBERG and E H RUBINSTEIN A proposed differentiated neuro-effector organization in muscle resistance vessels Angiologica 1964 1 197-208

FURNESS J B and M COSTA The adrenergic innervation of the gastrointestinal tract Reviews of Physiology Springer Verlag Berlin Heidelberg New York 1974 69 1 51

GILBERT P F C A theory of memory that explains the function and structure of the cerebellum Brain Res 1974 70 1-18

GLOOR P Amygdala In Handbook of Physiology Sect 1 Vol II American Physiological Society Washington 1960

GRANT R P LINDGREN A ROSEN and B UVNAS The release of catechols from the adrenal medulla on activation of the sympathetic vasodilator nerves to the skeletal muscles in the cat by hypothalamic stimulation Acta physiol scand 1958 43 135-154

GUREVITCH M I and A I VYSHATINA Cardiovascular responses to electrical stimulation of the fastigial nuclei Sechenov Physiol J USSR 1973 LIX 1715-1722

HATA N and M MIURA The inhibitory effect of cerebellar fastigial stimulation on ADH secretion J Physiol (Lond) 1974 242 793-803

HEATH R G and J W HARPER Ascending projections of the cerebellar fastigial nucleus to the hippocampus, amygdala and other temporal lobe sites: Evoked potential and histological studies in monkeys and cats Exp Neurol 1974 45 268-287

HESS W R und M BRÜGGER Der Miktions und der Defäkationsakt als Erfolg zentraler Reizung Helv Physiol Acta 1943 1 511 532

HOCKMAN C H K E LIVINGSTON and J TALESNIK Cerebellar modulation of reflex vagal bradycardia Brain Res 1970 23 101 104

HOFFER B J The effects of stimulation of the cerebellum on the circulatory system (Ph D Thesis) Univ of Rochester New York 1965

HOFFER B J R RATCHESON and R S SNIDER The effects of stimulation of the cerebellum on the circulatory system Fed Proc 1966 25 701

HULTEN L Extrinsic nervous control of colonic motility and blood flow Acta physiol scand 1969 Suppl 335

ITO M Neurophysiological aspects of the cerebellar motor control system Int J Neurology 1970 7 162 176

JANSEN J and A BRODAL Aspects on cerebellar anatomy Johan Grundt Tanum Forlag Oslo 1954

JANSSON G Extrinsic nervous control of gastric motility An experimental study in the cat Acta physiol scand 1969 a Suppl 326

JANSSON G Vago-vagal reflex relaxation of the stomach in the cat Acta physiol scand 1969 b 75 245-252

JANSSON G and J MARTINSON Studies on the ganglionic site of action of sympathetic outflow to the stomach Acta physiol scand 1966 68 184-192

JOHANSSON B O JONSSON and B LJUNG Supraspinal control of the intestino-intestinal inhibitory reflex Acta physiol scand 1965 63 442-449

KAPLAN P M Cerebellum and carbohydrate metabolism Bull Biol et Méd expér USSR 1938 V 212 214

KEWENTER J The vagal control of the jejunal and ileal motility and blood flow Acta physiol scand 1965 65 Suppl 251

KOCK N G A experimental analysis of mechanisms engaged in reflex inhibition of intestinal motility Acta physiol scand 1959 47 Suppl 164

KOELLA W P Motor effects from electrical stimulation of basal cerebellum in unrestrained cat J Neurophysiol 1955 18 559 573

KOPPANYI T Studies on defecation with special eference to a medullary defecation center J Lab Clin Med 1930 16 225-238

LANGHOF H U HÖPPENER and F J RUBIA Climbing fiber responses to spl nchnic nerve stimulation Brain Res 1973 53 232 236

LINDGREN P The mesencephalon and the vasomotor ystem Acta physiol scand 1955 35 Suppl 121

LISANDER B Factors influencing the autonomic component of the defence rea tion Acta physiol scand 1971 80 Suppl 351

LISANDER B I teractions among the defence reaction baroreceptor reflexes and cerebellar mechanisms In The nervous system in arterial hypertension Ed S Julius and M Esler C C Thomas Springfield Ill 1975 In press

LISANDER B and J MARTNER Cerebellar suppression of the autonomic components of the defence reaction Acta physiol scand 1971 a 81 84-95

LISANDER B and J MARTNER Interaction betwee the fastigial pressor response and the baroreceptor eflex Acta physiol scand 1971 b 83 505-514

LISANDER B and J MARTNER Influences on gastrointestinal and bladder motility by the fastigial nucleus Acta physiol scand 1974 90 792 794

MANCHANDA S K and R BHATTARAI Autonomic responses to stimulation of paleocerebellum-effects of intercollicular section Indian J Physiol Pharmacol 1972 16 329 338

MANCHANDA S K O P TANDON and I S ANEJA Role of the cerebellum in the control of gastro-intestinal motility J Neural Transmission 1972 33 195-209

MARR D A theory of cerebellar cortex J Physiol (Lond) 1969 202 437-470

MARTINSON J Studies on the efferent vagal control of the stomach Acta physiol scand 1965 65 Suppl 255

MITRA J and R S SNIDER Nucleus fastigii influences on blood flow and blood pressure Intern J Neuroscience 1972 3 285-290

MIURA M and D J REIS Cerebellum: a pressor response elicited from the fastigial nucleus and its efferent pathway in brainstem Brain Res 1969 13 595-599

MIURA M and D J REIS A blood pressure response from fastigial nucleus and its relay pathway in brainstem Amer J Physiol 1970 219 1330-1336

MIURA M and D J REIS The paramedian reticular nucleus: A site of inhibitory interaction between projections from fastigial nucleus and carotid sinus nerve acting on blood pressure J Physiol (Lond) 1971 216 441-460

MORUZZI G Ricerche sulla fisiologia del paleocerebellum I Riflessi labirintici e cervicali paleocerebellum e tono posturale Arch Fisiol 1936 36 57 112 (Cited in Dow and Moruzzi 1958)

MORUZZI G Azione del paleocerebellum sui riflessi vasomotori Arch Fisiol 1938 38 36-78 (Cited in Dow and Moruzzi 1958)

MORUZZI G Paleocerebellar inhibition of vasomotor and respiratory carotid sinus reflexes J Neurophysiol 1940 3 20-32

MORUZZI G Sham rage and localized autonomic responses elicited by cerebellar stimulation in the acute thalamic cat Proc XVII Internat Congress Physiol Oxford 1947 114-115

MORUZZI G Problems in cerebellar physiology C C Thomas Springfield Ill 1950

NATHAN M A Vasomotor projections of the nucleus fastigii to the medulla Brain Res 1972 41 194-198

NEWMAN P P and D H PAUL The representation of some visceral afferents in the anterior lobe of the cerebellum J Physiol (Lond) 1966 a 182 195-208

NEWMAN P P and D H PAUL The effects of stimulating cutaneous and splanchnic afferents on cerebellar unit discharges J Physiol (Lond) 1966 b 187 575-582

NEWMAN P P and D H PAUL The projection of splanchnic afferents on the cerebellum of the cat J Physiol (Lond) 1969 202 223-237

NORBERG K A Adrenergic innervation of the intestinal wall studied by fluorescence microscopy Int J Neuropharmacol 1964 3 379 382

OSKARSSON O Functional organization of spinocerebellar paths In Handbook of sensory physiology Vol II Ed A Iggo Springer Verlag Berlin Heidelberg New York 1973

OWSJANNIKOW P Arb aus d physiol Anstalt z Leipzig 1871 21 34 (cited by Wiggers 1943 a)

PETERS M and A A MONJAN Behavior after cerebellar lesions in cats and monkeys *Physiol and Behav* 1971 6 205–206

RAMU A and F BERGMANN The role of cerebellum in blood pressure regulation Experentia (Basel) 1967 23/5 383–384

RASHEED B M A S K MANCHANDA and B K ANAND Effects of the stimulation of paleocerebellum on certain vegetative functions in the cat Brain Res 1970 20 293–308

REIS D J and M CUENOD Central neural regulation of carotid baroreceptor reflexes in the cat Amer J Physiol 1965 209 1267–1277

REIS D J N DOBA and M A NATHAN Predatory attack grooming and consummatory behaviors evoked by electrical stimulation of cat cerebellar nuclei Science 1973 23 845–847

SAWYER C H J HILLIARD and T BAN Autonomic and EEG responses to cerebellar stimulation in rabbits Amer J Physiol 1961 200 405–412

SHERIDAN G and D J REIS Effects of cerebellar ablation on regional distribution of cardiac output in cat Brain Res 1972 45 260–265

SMITH O A and M A NATHAN Inhibition of the carotid sinus reflex by stimulation of the inferior olive Science 1966 154 674–675

STELLA G and G STEVAN Changes in the heart rate from stimulation of the cerebellar cortex in decerebrate dogs Arch *Int Pharmacodyn* 1962 136 1 11

THOMAS D M R P KAUFMAN J M SPRAGUE and W W CHAMBERS Experime tal studies of the vermal cerebellar projections in the brain stem of the cat (fastigiobulbar tract) J Anat (Lond) 1956 90 371 385

WADA M M SEO and K ABE Effect of the i jury of the lobulus posterior medianus (Ingvar) of the cerebellum upon the blood pressure the blood sugar content and the epinephrine secretion in non-anaesthetized non-fastened dogs Tohoku J exp Med 1935 27 218–244

WHITESIDE J A and R D GUYTON Cerebellar influence on bladder musculature Anat Rec 1952 112 401

WIDEN L Cerebellar representation of high threshold afferents in the splanchnic nerve with observation on the cerebellar projection of high threshold somatic afferent fibres Acta physl I scand 1955 33 Suppl 117

WIGGERS K The influence of the cerebellum on the vegetative functions I A review Arch néerl de physiol 1943 a 27 286-300

WIGGERS K The influence of the cerebellum on the heart and circulation of the blood II Arch néerl de physiol 1943 b 27 301 303

WISE R.A Spread of current from monopolar stimulation of the lateral hypothalamus Amer J Physiol 1972 223 545-548

WOLFE J W., Chronic gastric ulceration associated with experimentally induced posterior cerebellar vermal lesions Physiol Behav 1969 4 1011 1013

VORONIN L G New data on the motor activity of intestinal tract and on its regulatory mechanism Izv naucn Inst Lesgafta 1938 21 3 74 (English summary 72 74)

VORONIN L G and A M SIMKINA The influence of electrical stimulation of the cerebellum on the motor function of the intestinal tract Izv naucn Inst Lesgafta 1938 21 75-85

YOUMANS W B Innervation of the gastrointestinal tract In Handbook of physiology Sect 6 Vol 4 American Physiological Society Washington 1968

ZANCHETTI A and A ZOCCOLINI Autonomic hypothalamic outbursts elicited by cerebellar stimulation J Neurophysiol 1954 17 475-483

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 426

Extrinsic Nervous Control of the Ileo-cecal Sphincter in the Cat

BY

PER ERIK PAHLIN

GÖTEBORG 1975

ACTA PHYSIOLOGICA SCANDINAVICA
SUPPLEMENTUM 426

FROM THE DEPARTMENT OF SURGERY III, SAHLGREN'S HOSPITAL,
UNIVERSITY OF GÖTEBORG, GÖTEBORG, SWEDEN

Extrinsic Nervous Control of the Ileo-cecal Sphincter in the Cat

BY

PER ERIK PAHLIN

GÖTEBORG 1975

This thesis is mainly based on the following papers which will be referred to in the text by their Roman numerals

I. Pahlin, P E. and J Kewenter The direct sympathetic nervous control of the cat ileo-cecal sphincter *Amer J Physiol*. Submitted for publication.

II Pahlin, P E. and J Kewenter Adrenoceptors in the cat ileo-cecal sphincter and intestine studied *in vivo* *Amer J Physiol* Submitted for publication.

III. Pahlin, P E. and J Kewenter The vagal control of the ileo-cecal sphincter in the cat. *Acta physiol scand.* Submitted for publication.

IV Pahlin, P E. and J Kewenter Reflexogenic contraction of the ileo-cecal sphincter in the cat following small or large intestinal distension. *Acta physiol. scand* In press.

KUNGSBACKA 1975
ELANDERS BOKTRYCKERI AKTIEBOLAG

CONTENTS

INTRODUCTION 5
- Anatomy and function 5
- The sympathetic and parasympathetic innervation of the ICS 7
- The autonomic transmitter mechanism 7
- Reflex activation of the ICS 8

AIMS OF THE INVESTIGATION 9

MATERIAL AND METHODS 10
- Operative procedure 10
- Recording of transsphincteric flow 10
- Recording of opening pressure 12
- Recording of intestinal motility 12
- Nerve stimulation 12
- Intestinal distension 12
- Nerve sections 12
- Spinal anesthesia 12
- Drugs 13

METHODOLOGICAL CONSIDERATIONS 14

RESULTS AND COMMENTS 16
- Sympathetic nerve stimulation 16
  - Autonomic blockade 16
- Vagal nerve stimulation 20
  - Autonomic blockade 21
- Termination of adrenergic fibres in the ICS 22
- Reflex activation of the ICS by intestinal distension 25
  - Nerve section and spinal anesthesia 25
  - Autonomic blockade 26

FUNCTIONAL CONSIDERATIONS 27

SUMMARY 28

ACKNOWLEDGEMENTS 28

REFERENCES 29

INTRODUCTION

Anatomy and function

The gastrointestinal sphincters separate portions of the alimentary canal which differ in anatomy and physiology. An adequate sphincter function can play an important role in preventing pathological conditions, both in the digestive tract and in the body as a whole.

A sphincter is a muscle which surrounds and serves to close an orifice. This definition covers the major functions of the gastrointestinal sphincters i.e. to keep the gastrointestinal contents in one part of the alimentary canal for an appropriate time by preventing too rapid passage or preventing regurgitation. The sphincter of Oddi has a specific function by regulating the bile flow to the intestine but also prevents regurgitation of duodenal contents into the bile ducts.

The gastrointestinal sphincters cannot always be identified anatomically e.g. the gastrooesophageal sphincter in man (Higgs *et al.* 1965). They might, however be identified by some of their characteristics and different definitions of these characteristics have been proposed. Thus, some physiological characteristics of a gastrointestinal sphincter were defined by e.g. Cohen *et al.* (1968) and Fisher and Cohen (1973): *a)* A sphincter exhibits an intraluminal pressure greater than that of the cavities separated by the sphincter. *b)* Appropriate stimulation proximal to the sphincter results in a consistent fall in the elevated pressure within the sphincter. *c)* Appropriate stimulation distal to the sphincter results in a prompt rise of the sphincteric pressure. Pharmacologically the gastrointestinal sphincters have been characterized *in vitro* by Bass *et al.* (1970). Sphincteric tissue contracts with adrenaline whereas non sphincteric tissue does not. Thus, although a gastrointestinal sphincter cannot always be identified anatomically there are physiological or pharmacological characteristics by which they can be defined.

The first anatomical description of the ileal termination into the colon was made by Variolus in 1573, but it was Bauhin whose name became associated with this anatomical structure although he did not describe it until 6 years after Variolus. (For historical notes see DiDio and Andersson 1968). However it was Bauhin who, when describing the ileal termination into the colon, was the first to use the term "valvula" which is defined as a membranous fold in a canal or passage which serves to prevent backward reflux of the contents. The concept of an "ileo-cecal valve" dates back to Bauhin's description. It was not until the classical study by Elliott (1904) that a sphincteric function was attributed to the ileo-cecal junction.

Earlier investigations were made in cadavers. When the ileo-cecal junction was studied in living subjects there were no signs of any membranous folds. In the cat the ileal termination in the colon has a similar appearance as in man. Thus, in both the cat and in man the terminal ileum protrudes into the colon and is therefore surrounded by the colonic wall (Elliott 1904 Rosenberg and DiDio 1969) Elliott (1904) investigated the anatomical arrangement of the ileo-cecal junction of the cat and found that the circular muscular coat of the ileum was considerably thickened over about the last 10 mm of its course whereas the longitudinal layer was very thin. Gazet (1968) compared the combined thickness of the circular muscle of the normal ileum and colon with that of the ileo-cecal junctional zone in man, the cat, the dog and the rhesus-monkey In man, the cat, and the dog there was a considerable thickness of the circular layer in the ileo-cecal junctional zone, but in the rhesus monkey no difference was found.

As mentioned, Elliott (1904) was the first to point out from physiological studies in cats, that the ileo-cecal junction was guarded by a sphincter In recent years Kelley Jr *et al* (1966) and Cohen *et al* (1968) have confirmed that the ileo-cecal junction in the dog and in man respectively have the physiological characteristics of a gastrointestinal sphincter This has also been shown pharmacologically *in vitro* in several species e.g. the cat, the guinea-pig, the rabbit the dog, the rhesus monkey and man (Munro 1951 Reddy 1960 Gazet and Jarrett 1964 Bass *et al* 1970)

According to Alvarez (1948), the ileo-cecal sphincter (ICS) has two purposes: firstly to prevent reflux from the colon into the ileum and secondly to prevent too rapid passage of food residues through the terminal segment of the small bowel.

Two different mechanisms for a reflux preventing function of the ICS have been discussed a pure valvular mechanism (Hammer 1927 Friedell and Wakefield 1941 Fleischner and Bernstein 1950) and a sphincteric mechanism (Elliott 1904 Katsch 1913 Gazet 1968, Cohen *et al* 1968) Other investigators have considered both mechanisms to be the cause of the competence of the ileo-cecal junction in preventing reflux (Hromada 1921 Ulin *et al* 1956, Lenz 1964).

A reflux of barium into the ileum during the course of a barium enema can be accomplished in about 90% of patients (Fleischner and Bernstein 1950, Miller 1965). Rendleman *et al* (1958) found that there was an increasing incompetence with increasing pressure of the enema. These findings can hardly be explained if there is a valvular effect. Increasing pressures would then by definition close the valve more tightly A sphincteric mechanism accords better with the above findings. The sphincter yields to the pressure in the colon. During barium enema the pressure is usually high and may exceed 90 cm H_2O This might explain the high degree of sphincter incompetence found in these studies. Under physiological conditions the pressure rarely exceeds 50 cm H_2O (Kock *et al* 1968).

Whether the ICS also serves to regulate the entrance of the ileal contents into the colon is not quite clear Kaiser *et al* (1960) and Stahlgren *et al* (1962) considered the ICS to be of great importance for the normal small intestinal transit time.

Singleton *et al.* (1964) however were able to register only a slight decrease of the transit time by excision of the ICS or 50% of the small intestine, but when both parts were excised there was a marked decrease in the average transit time. Similarly DiDio and Anderson (1968) considered the combination of the motility in the distal ileum and the resistance to flow offered by the ICS to be important for the regulation of the passage of the gastrointestinal contents from the ileum into the cecum.

The sympathetic and parasympathetic innervation of the ICS

Elliott (1904) found that the removal of the spinal cord permanently abolished the ability of the sphincter to keep the contents of the ileum and the colon apart, indicating that the sphincteric muscles are dependent upon an intact extrinsic nervous system for adequate function. However, Tönnis (1924) considered that the exogenous nerves to the ICS exerted a regulatory control on an autonomically functioning sphincter but that the sphincter could function although these nerves had been cut.

From previous studies on the extrinsic nervous regulation of the ICS it is known that stimulation of *the splanchnic nerves* elicits an increase of pressure within the ileo-cecal junction in cats and dogs due to contraction of the ICS (Elliott 1904 Dale 1906, Hinrichsen and Ivy 1931 Jarrett and Gazet 1966). *The lumbar colonic nerves* which are mainly postganglionic fibres of the lumbar sympathetic outflow have not been shown to take part in the innervation of the ICS. Elliott (1904) found no changes in the tone of the ICS upon stimulation of these nerves.

The influence of *the vagal nerves* upon the ICS has been sparsely investigated. Elliott (1904) discussed the possibility of the contraction of the ICS following cervical vagal stimulation being due to anaemia in the intestine caused by the concomitant inhibition of the heart. Hinrichsen and Ivy (1931) recorded in dogs a diphasic response following electrical stimulation of these nerves; relaxation followed by contraction. In cats, Jarrett and Gazet (1966) noted different responses depending upon the frequency of the stimulation, contraction was elicited at high frequencies (50 imp/s) and relaxation at low frequencies (5 imp/s) In dogs, however only contractions were recorded. As the vagal nerves convey excitatory fibres to the proximal colon (Stavney *et al* 1963 Hultén 1969) it cannot be excluded that the vagally induced contraction of the ICS might be partly or entirely due to a secondary squeezing effect of the colonic wall surrounding the sphincter. Thus, the influence of the vagal nerves upon the ileo-cecal sphincter muscle must be considered unclear

The autonomic transmitter mechanism

The autonomic transmitter mechanism involved in the responses of the ICS to stimulation of the sympathetic and parasympathetic nerves has been little studied. The classical concept of the sympathetic system beeing strictly adrenergic and the

parasympathetic one strictly cholinergic has been shown to have many exceptions (see Christensen 1968). Thus the sympathetic nerves may contain cholinergic fibres (Thomas and Baldwin 1968, Hultén 1969 Persson 1973) and the parasympathetic nerves adrenergic fibres (Martin *et al* 1974). Furthermore, it has been clearly demonstrated that motor responses obtained in the gastrointestinal tract following autonomic nerve stimulation can be elicited by a non-cholinergic, non-adrenergic mechanism (for review see Burnstock 1972).

The work of Dale (1906) indicated an adrenergic mechanism as the cause of contraction of the ICS following splanchnic nerve stimulation in the cat. Studies *in vitro* on muscle strips from the ICS in different species (rabbit, guinea pig, cat, dog and man) have shown that these strips contract with both adrenergic and cholinergic drugs (Reddy 1960 Reynolds *et al* 1967 Gazet and Jarrett 1964 Bass *et al.* 1970). This indicates adrenergic as well as cholinergic excitatory receptors within the ICS. It has been shown, also *in vitro* that the excitatory response to the adrenergic drugs was mediated by α-receptors (Reddy 1960, Gazet and Jarrett 1964 Reynolds *et al.* 1967). Inhibitory adrenergic β-receptors in the ICS were postulated from *in-vitro* studies in cats, dogs, man and rhesus-monkeys by Gazet and Jarrett (1964) and in guineapigs by Reynolds *et al* (1967). Reddy (1960) found that isoprenaline in small doses relaxed muscle strips from the rabbit ICS while large doses elicited a contraction. Both effects were blocked by β receptor blockade, indicating that both relaxation and contraction could be mediated *via* the β-receptors. In the small and large intestines the α-receptors are considered generally to be inhibitory (Ahlquist and Levy 1959 Furchgott 1960 Brody and Diamond 1967) A difference in function *in vitro* of the α-receptors in the ICS and the intestinal smooth muscles is thus indicated. Therefore a further study *in vivo* of the functional significance of these *in-vitro* results seemed to be justified.

Reflex activation of the ICS

Reflex activation of the ICS by distension of different parts of the intestinal tract has been reported. Hinrichsen and Ivy (1931) noted contractions upon distension of the stomach and the small and large intestines in dogs while Chang and Hsu (1942) upon distension of small intestinal loops in dogs, recorded both contractions and relaxations in the sphincteric area depending upon the tone of the sphincter at the time of the distension. The former authors considered that the reflex was dependent upon both the splanchnic and vagal nerves being intact while the latter authors considered the splanchnic nerves to be the main pathway for the reflex.

AIMS OF THE INVESTIGATION

From previous studies it may be concluded that the junctional zone between the ileum and the colon is guarded by a sphincter. Only a few studies have dealt with the extrinsic nervous control of the sphincter and the results are sometimes conflicting. Furthermore, few data concerning the autonomic mechanisms involved in the nervous control of the ICS are available.

The aims of the present investigation were therefor to study:

1. The direct splanchnic and lumbar colonic nervous control of the ICS.
2. The adrenergic α-and β receptor mechanisms in the ICS.
3. The direct vagal control of the ICS
4. The nervous pathways and peripheral autonomic transmitter mechanism involved in the reflex activation of the ICS following intestinal distension.

MATERIAL AND METHODS

Experiments were performed on 181 adult cats of both sexes. The number of animals used in the various series is given in each paper. Some cats were used for experiments in several different series. The cats were fasted for about 24 hours before the experiments, but were allowed free access to water. In all animals anesthesia was induced with ether and maintained by an intravenous injection of chloralose (50 mg/kg b.w.). A tracheal cannula was inserted to maintain free airways and all animals breathed spontaneously except in a few experiments (IV) in which a respirator was used. The blood pressure was continuously recorded by means of a mercury manometer connected to a femoral artery. Great care was taken to keep the animal at normal body temperature.

Operative procedure. Laparotomy was performed through midline incision. In most cats the small intestine, apart from the parts used for distension (IV) and for recording intestinal motility, was extirpated. The colon was left in place. As catecholamine secretion from the adrenal medulla is known to be a potent intestinal inhibitor (Kock 1959) the vessels of these glands were ligated in the beginning of all experiments except for two (IV) in which ligatures were performed during the course of the experiments.

Recording of transsphincteric flow (I—IV). Fig. 1 shows a schematic drawing of the method for recording transsphincteric flow. The ileum was divided about one cm proximal to the sphincter and a "crushing" ligature was tied around the colon one to two cm distal to the sphincter. In this way the ileo-cecal region was isolated from the adjacent gut but with the mesenteric vascular and nerve supply intact. A tube was introduced into the remaining ileum distal to the transection and fixed with a ligature at the oral end of the sphincter. Normal saline, thermostatically regulated to be at body temperature, was led from a reservoir to the sphincter *via* the tube. The saline passing the sphincter was drained from the cecum by means of a funnel introduced with its wider end through an incision in the antimesenteric border of the gut opposite to the sphincter. The narrow end of the funnel was connected to a Gaddum recorder (Gaddum 1929). Volume changes in the recorder reflected changes in the flow through the sphincter. The volumes were registered *via* a piston-recorder on a smoked drum. From the Gaddum recorder the saline was

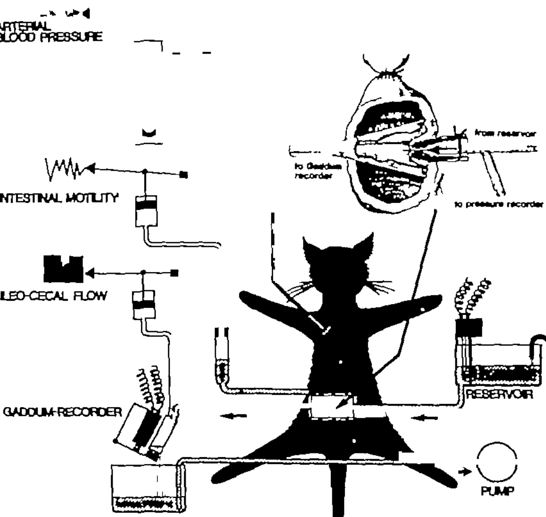


Fig. 1 Schematic drawing of the experimental set up. For explanation see text. (Pressure recorder not illustrated in the figure).

continuously recirculated into the reservoir. As the reservoir had a large cross-sectional area the inflow pressure, i.e. the height of the saline level over the sphincter could be kept almost constant. The inflow pressure was usually kept around 5 cm H_2O at which pressure the flow capacity of the inflow tube was 3—4 times greater than the maximal flow recorded through the sphincter. The reservoir could be adjusted to different heights so the sphincter could be perfused at different pressures.

Recording of opening pressure (I and III) The opening pressure was defined as the pressure needed to induce a flow through a closed sphincter This pressure was measured from a small side-branch to the perfusion tube just proximal to the sphincter (Fig. 1) and was registered *via* a pressure receptor on a kymograph (Mingograph, Siemens-Elema, Stockholm) When the sphincter was closed, the perfusion pressure was raised by elevating the reservoir until the flow just started. The pressure recorded at that moment was thus called the opening pressure.

Recording of intestinal motility (I—IV) The small and large intestinal motility was recorded with a volume recording technique previously described (e.g. Kock 1959). About 10 cm long intestinal loops from the proximal jejunum, the ileum just proximal to the ICS and the colon just distal to the ICS were studied. The loops were isolated from the adjacent gut but with their mesenteric nerve and blood supply intact.

Nerve stimulation. (I—IV) The following nerve trunks were divided and stimulated electrically in the efferent direction. the two major splanchnic nerves subdiaphragmatically (I—IV) the nerves along the superior mesenteric artery here called "the periarterial nerves" (I and II) the nerves along the inferior mesenteric artery known as "the lumbar colonic nerves (e.g. Garry 1933) (I and II) and the cervical vagal nerves (III).

Square wave pulses were delivered from a Grass stimulator (S4E). Supramaximal currents were used (4—12 V 3—5 ms) 4—8 imp/s was usually used but the frequency was varied over a wide range (2—50 imp/s) in some experiments. Stimulation trains between 30—60 seconds were usually used.

Intestinal distension. (IV) Intestinal distension was elicited from a jejunal, ileal or colonic loop of about 10—15 cm in length. The loops were isolated from the adjacent gut but with the mesenteric nerve and vascular supply intact. The loops were distended with body-warm saline from a reservoir that could be adjusted to different heights. Distensions of 30—60 seconds duration and at intervals of 10 minutes were usually applied. The pressures used were 30—100 cm H_2O

Nerve sections (IV). To elucidate the possible nervous pathways for the reflex activation of the ICS upon intestinal distension the following nerves were cut: the vagal nerves at cervical level the pelvic nerves at the outlet from sacrum the two major splanchnic nerves subdiaphragmatically the lumbar colonic nerves along the inferior mesenteric artery

Spinal anesthesia. (IV) In order to achieve a preganglionic sympathetic blockade, spinal anesthesia was performed. The anesthetics were injected *via* a thin polyethylene catheter introduced intradurally in the caudal direction through a cervical laminectomy

Drugs. (I—IV) Drugs were administered to produce blockade as follows. muscarinic" cholinergic receptors. atropine (Atropine sulphate, Merck) 0 1—1 mg/kg b.w i.v (I, II, III and IV).
Ganglionic transmission. chlorisondamine (Ecolid® Ciba) 2 mg/kg b.w i.v (I)
Adrenergic transmission. guanethidine (Ismelin® Ciba) 1—5 mg/kg b.w i.v (I, III and IV)
Adrenergic α-receptors: phenoxybenzamine (Dibenyline® Smith, Klein and French) 1—15 mg/kg b.w i.v (II and IV).
Adrenergic β-receptors: propranolol (Inderal® ICI) 1—2 mg/kg b.w i.v (II and IV).
The following sympathomimetic drugs were used adrenaline 0 1—2 γ/kg b.w., noradrenaline 0.1—7 γ/kg b.w., phenylephrine 0.3—11 γ/kg b w., and isoprenaline 2—100 γ/kg b.w These drugs were administered either intravenously through a femoral vein or intraarterially in the retrograde direction through a small branch to the superior mesenteric artery (II and III).
Spinal anesthesia (IV) was induced with Tetracaine grave® (Apoteksbolaget, Sweden) *10 mg/ml or mepivacaine chloride (Carbocain® Nobel Pharma) 20 mg/ml.* Both drugs were administered in an amount 1—2 ml intradurally

METHODOLOGICAL CONSIDERATIONS

In previous studies in which the extrinsic nervous control of the ICS has been investigated pressure registration techniques have usually been used (Elliott 1904 Hinrichsen and Ivy 1931 Jarrett and Gazet 1966) However when pressure receptors are used it may be difficult to keep the receptor within the sphincteric region (Elliott 1904) Furthermore, the ICS has a flow regulating function, and it was therefore thought of interest to use a technique by which the flow through the sphincter could be studied The method described in the present report was suitable for this purpose (I) Changes in the transsphincteric flow could be recorded continuously throughout the whole experiment. The changes of the flow at a constant perfusion pressure were due to changes in the tone of the ileo-cecal junction as the inflow tube could easily be placed at the proximal end of the sphincter Furthermore, the perfusion tube and the funnel used to drain the ICS had a flow capacity which was 3—4 times larger than the maximal flow recorded through the ICS at an inflow pressure of about 5 cm H_2O

The perfusion pressure could be kept constant as the cross-sectional area of the saline reservoir was large and as the saline was continuously recirculated. The pressure was usually set to about 5 cm H_2O At this pressure the flow through the sphincter was easily maintained. The normal resting pressure within the cat ileo-cecal junction is not known. In unanesthetized dogs (Kelley Jr *et al.* 1965) and in man (Cohen *et al* 1968) the average resting pressure was as high as 19 cm H_2O and 20 mm Hg respectively above the ambient intestinal pressure. Provided that there are only minor species differences in the resting pressure, the tone within the ICS in the present experiments was comparably low The cause of this low tone is unclear However anesthesia has been shown to reduce the pressure greatly within the lower oesophageal sphincter (Code and Schlegel 1968) and in the pharyngo-oesophageal sphincter (Levitt *et al* 1965) This might also be the cause of the low tone of the ICS in the present studies. As the flow pattern was the same whether the extrinsic nerves were intact or not, influence from these nerves does not seem to be the cause of the low sphincteric tone.

The temperature of the perfusion saline was of great importance. If the temperature was set at about +20°C a high perfusion pressure (sometimes about 50 cm H_2O) had to be used to maintain a transsphincteric flow while with the saline at body temperature the flow could easily be maintained at a low perfusion pressure (about 5 cm H_2O)

As previously stated, the terminal ileum protrudes into the colon and is thus surrounded by the colonic wall (Elliott 1904) Changes in the transsphincteric flow might therefore depend upon changes either in the tone of the sphincter itself, the tone of the colon, or both. However, the motor activity of the intestines just proximal and distal to the sphincter was recorded simultaneously with the transsphincteric flow in some of the experiments in order to compare the motor activity in the intestine and the ICS

RESULTS AND COMMENTS

Sympathetic nerve stimulation (I—IV)

Efferent electric stimulation at physiological frequencies (2—12 imp/s) and supramaximal currents of the distal ends of the divided major splanchnic nerves, the periarterial nerves or the lumbar colonic nerves elicited reduction or inhibition of the transsphincteric flow concomitant with an inhibition of the ileal and proximal colonic motility. As the flow reduction was noted within a few seconds and any influence from the suprarenal glands was excluded, it was concluded that the effect of sympathetic nerve stimulation was due to an excitatory nervous effect on the ICS. In some of the experiments a slight increase of the transsphincteric flow could be recorded during the first few seconds of stimulation. This flow increase could not be regularly elicited although the stimulation parameters were varied over a wide range. The results indicate that the main influence of the sympathetic nerves on the ICS is excitatory which is in accordance with most previous studies (Elliott 1904, Dale 1906, Hinrichsen and Ivy 1931, Jarrett and Gazet 1966). Excitatory fibres within the lumbar colonic nerves to the ICS seem not to have been previously reported.

Autonomic blockade (I—II). The splanchnic, periarterial and lumbar colonic nerves were also stimulated after intravenous administration of atropine (0.2 and 1 mg/kg b.w.) to investigate whether ICS contraction was due to an activation of cholinergic fibres within these nerves (I). The lower dose of atropine did not block the excitatory effect of the ICS to the nerve stimulation, while the larger dose caused a transient blockade of the response. A similar transient blockade by atropine (1 mg/kg b.w.) of the excitatory effect of noradrenaline on the ICS was also found in 6 cats investigated (Fig. 2). After 15—60 minutes, splanchnic nerve stimulation, as well as noradrenaline infusion, elicited the same response on the ICS as before atropine had been given. A similar antagonistic effect of atropine on the excitatory response in the sphincter of Oddi to sympathetic nerve stimulation *in vivo* in the cat has been reported by Persson (1973) and in the lower oesophageal sphincter by Clark and Vane (1961). Furthermore, Christensen and Daniel (1968) found that atropine blocked the excitatory response to noradrenaline in an *in-vitro* preparation from the smooth muscle of the lower oesophageal sphincter.

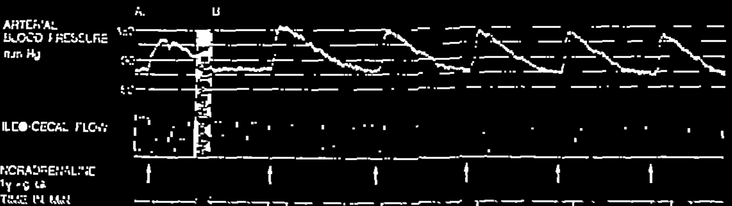


Fig 2. Effect of intra-arterial injection of noradrenaline on the transsphincteric flow before and after atropine. Note that a transient blockade of the response was obtained.

This blocking action of atropine is different from the muscarinic effect of the drug causing the blockade of the excitatory response in the stomach (Martinson 1965) the small and large intestines (Kewenter 1965 Hultén 1969) and the ICS (III) by vagal nerve stimulation. In these experiments, even small doses (0.1—0.5 mg/kg b w) of atropine blocked the effect of vagal stimulation for several hours. It does not seem likely therefore, that the short lasting suppressant effect of large doses of atropine on the excitatory response of the ICS following sympathetic nerve stimulation is due to an anticholinergic mechanism. Non specific effects of atropine in the higher dose (1 0 mg/kg b.w i.v) might be caused by an antiadrenergic effect of the d isomer of hyoscyamine in atropine as reported by Luduena and Branin (1966) Furthermore, atropine in large doses is also known to have a slight ganglion-blocking action (Marrazzi 1939) As the response in the ICS to periarterial and lumbar colonic nerve stimulation, which nerves are considered to be mainly post ganglionic, was reduced in the same way as the effect of splanchnic nerve stimulation this latter explanation seems not to be relevant.

The effect of autonomic blocking drugs on the initial slight increase of the transsphincteric flow at splanchnic stimulation could not be evaluated due to the irregularity in this response. As the responses were also seen after atropine, however the flow increase seems not to be dependent on a cholinergic mechanism.

Guanethidine completely blocked the excitatory response of the ICS to sympathetic nerve stimulation, which indicates an adrenergic transmitter mechanism (I) This was further supported by the results reported in paper II, in which it was shown that adrenaline, noradrenaline and phenylephrine also elicited a contraction of the ICS. The sphincteric excitatory responses obtained by both the sympathomimetic amines and sympathetic nerve stimulation were blocked by the α-receptor blocking agent phenoxybenzamine, while they remained after β receptor blockade.

After propranolol the excitatory response in the ICS following injection of sympathomimetic amines (adrenaline, noradrenaline, phenylephrine) was enhanced in more than 2/3 of the experiments (II) Following adrenergic nerve stimulation a corresponding increase of the sphincter contraction after propranolol was seen in about 1/3 of the experiments (II). The increased response might be due to an unmasking of the excitatory α-receptor response through blockade of inhibitory β receptors by propranolol This effect of β blockers has been proposed by Ahlquist (1968) Inhibitory β receptors could be further established in a series of experiments showing that isoprenaline relaxed the ICS during a sphincter contraction elicited by adrenergic nerve stimulation (II) This increased transsphincteric flow following isoprenaline was not due to relaxation of the intestine surrounding the sphincter as the intestine was already relaxed by the adrenergic nerve stimulation. The inhibitory effect of isoprenaline was blocked by propranolol. These studies strongly suggest the presence of inhibitory β-receptors in the ICS.

The inhibition of intestinal activity elicited by adrenergic stimulation is mediated *via* both α- and β receptors (Ahlquist and Levy 1959 Furchgott 1960 Bucknell

and Whitney 1964 Brody and Diamond 1967). In paper II it was shown that neither phenoxybenzamine nor propranolol blocked the inhibitory motor response of the small and large intestinal loops following adrenergic nerve stimulation. Both an α- and β-receptor blocker had to be administered to obtain complete blockade of the inhibitory motor response elicited by nerve stimulation. This confirms the concept of both α- and β receptors as mediators of adrenergic inhibition of the intestine. *A functional difference between the α-receptors in the ICS and the intestine* is thus indicated.

From *in-vitro* studies on the guinea pig ileum Kosterlitz and Watt (1965) suggested that the inhibitory α-receptors are located within the intramural cholinergic ganglion cells while the inhibitory β receptors are situated on the muscle cell. If the effect of the adrenergic nerves on intestinal motor activity is confined exclusively or almost exclusively to the cholinergic ganglion cells, as suggested by histochemical and physiological studies (Norberg 1964 Jacobowitz 1965 Kewenter 1965 Jansson and Martinson 1966, Hultén 1969), one would then expect that the inhibitory response of the intestine, following sympathetic nerve stimulation, would be blocked by phenoxybenzamine only provided that the suggestion by Kosterlitz and Watt (1965) is true. The fact that such a blockade was not obtained might be explained by e.g. direct adrenergic fibres to the intestinal smooth muscle eliciting inhibition *via* unblocked β-receptors. Adrenergic fibres within the circular smooth muscles of the intestine have been demonstrated histochemically in recent years by e.g. Costa and Gabella (1971) and Silva *et al* (1971) Another possibility might be an overflow of the transmitter from adrenergic nerve endings to the β receptors at the muscle cells (Paton and Vizi 1969). A third possibility is that the α-receptors within the intestine have not been completely blocked although a total blockade of the effect on the blood pressure and sphincter following phenoxybenzamine administration was indicated. Irrespective of the mode of action it may be concluded that the α-receptors within the ICS and the intestine produce a different response, contraction and inhibition respectively when they are activated by means of splanchnic or lumbar colonic nerve stimulation. Furthermore, the present results indicate that the inhibitory adrenergic influence on the motor activity of the small intestine is not totally mediated *via* α-adrenergic receptors on the intramural cholinergic ganglion cells.

In general, a sympathetically innervated organ has a preponderance of receptors *of one type, although a small* proportion of the other type may also be present (Innes and Nickerson 1968). In this study contraction of the ICS was the dominating response to sympathetic nerve stimulation as well as to catecholamine infusion which responses were blocked by phenoxybenzamine. This indicates that excitatory α-receptors are in preponderance within the ICS.

To summarize the experiments in I and II have shown that sympathetic nerve stimulation as well as adrenaline, noradrenaline and phenylephrine elicits a contraction of the ICS. As this response was *blocked by* guanethidine and phenoxy-

benzamine but not by propranolol an adrenergic α-receptor mechanism as cause of the response is strongly indicated. The presence of inhibitory β-receptors within the ICS is also suggested. Both phenoxybenzamine and propranolol had to be administered to block the adrenergic inhibitory effect on the intestinal motor activity

Vagal nerve stimulation (III)

Efferent cervical vagal nerve stimulation with physiological frequencies and supra maximal currents was shown to reduce or abolish the transsphincteric flow In contrast to the effect of sympathetic stimulation this flow decrease was usually concomitant with an increase in motor activity in the small intestine and more seldom in the proximal part of the large bowel. The reduced transsphincteric flow might thus be due either to a contraction of that part of the intestine that surrounds the sphincter or to a contraction of the sphincter itself or both. To elucidate whether the decreased transsphincteric flow was due to sphincteric contraction or not, the vagal stimulation was repeated during continuous sympathetic nerve stimulation, which is known to suppress the excitatory effect of vagal stimulation on the intestine (Kewenter 1965 Hultén 1969). It was then found that vagal stimulation stopped the transsphincteric flow although no concomitant ileal contraction could be recorded. There were thus indications that the flow stopped because of a contraction of the sphincteric muscle.

The hypothesis of an excitatory vagal nervous effect on the sphincteric muscle was further supported by the results obtained with vagal stimulation after administration of guanethidine. Before this drug an increased motor activity in the colon was only obtained in a few experiments following vagal stimulation, while there was a stop of the transsphincteric flow in the majority of the experiments. In contrast, after guanethidine vagal stimulation elicited a pronounced contraction of the colon in the majority of the experiments but no or a delayed, reduction of the transsphincteric flow It therefore seems unlikely that the colonic motor activity interferes with the transsphincteric flow It can of course be argued that the colonic motor activity was recorded distal to the sphincter region but there are no indications of a qualitative difference in the influence of the vagal nerves on the cecum and the proximal part of the colon.

In order to reveal any inhibitory fibres within the vagal nerves to the ICS stimulation was repeated during continuous intra arterial infusion of noradrenaline in order to create an increased sphincter tone on which possible inhibitory fibres could act. The opening pressure was repeatedly recorded in this series of experiments. As shown in Fig. 3 the opening pressure gradually decreased despite continuous noradrenaline infusion. When the vagal nerves were stimulated the opening pressure increased once again. A decrease of opening pressure upon vagal stimulation was never obtained, indicating that no relaxatory fibres are present in the vagus to the ICS.

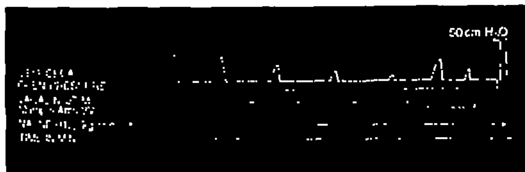


Fig. 3. Effect of vagal nerve stimulation on the opening pressure during intra-arterial noradrenaline infusion. Note the gradually decreasing opening pressure despite the continuous infusion. Vagal stimulation again elicited an increased opening pressure.

Catecholamines have been shown to suppress the output of acetylcholine in *in-vitro* preparations from the colon and ileum subjected to nerve stimulation (Beani *et al.* 1969, Paton and Vizi 1969) and to suppress the intestinal excitatory motor response following vagal stimulation *in vivo* (Kewenter 1965). The increase in opening pressure upon vagal stimulation during noradrenaline infusion therefore supports the assumption that the vagal nerves exert their effect direct on the ICS and not *via* the colonic wall surrounding the sphincter.

Increased pressure in the ileo-cecal junction of the dog following vagal nerve stimulation was also obtained by Hinrichsen and Ivy (1931). In their experiments the contraction was usually preceded by a slight inhibition of the sphincteric tone, an effect that was never observed in the present experiments. In the cat, Jarrett and Gazet (1966) could elicit different responses in the ICS by cervical vagal stimulation. They obtained relaxation at low frequencies (5 imp/s) and contraction at high frequencies (50 imp/s). In the present study only excitatory responses were obtained at frequencies between 2 and 50 imp/s.

Autonomic blockade. Atropine and guanethidine are widely used to block cholinergic and adrenergic nervous transmission respectively. In paper III it was found that atropine blocked all the responses to vagal stimulation but also that guanethidine in most experiments blocked or markedly delayed the contractile response of the ICS to vagal stimulation, while the excitatory effect on the intestinal loops was greatly enhanced.

The results concerning the blockade by both drugs of the vagally induced sphincteric response are difficult to explain. Guanethidine has been shown to have an anticholinergic action in certain nerve-muscle preparations (Boyd *et al.* 1963) while atropine in low doses is considered to be a specific anticholinergic agent (Innes and Nickerson 1968). Therefore a cholinergic mechanism seems to be the most plausible cause of the contraction of the ICS upon vagal stimulation.

According to Burnstock (1972) non-adrenergic inhibitory as well as non-adrenergic, non-cholinergic excitatory neurons are present in the small intestine of mammals. In the present study neither excitation nor inhibition of the ICS was obtained after atropine and/or guanethidine upon vagal stimulation. Therefore no indications of the presence of such neurons in connection with the vagal nerves have been obtained.

From the results in III it can be summarized that vagal nerve stimulation causes a decrease of the transsphincteric flow depending, at least partly upon a direct excitatory nervous effect on the ICS muscle. This effect was suggested to be cholinergic.

Termination of adrenergic fibres in the ICS

Excitatory responses in the ICS obtained by both cholinergic and adrenergic nerve stimulation give rise to several alternatives in the mutual relationship between the adrenergic and cholinergic nerve terminals within the intestinal wall. This mutual relationship has previously been discussed (e.g. Christensen 1968, Christensen 1971 Youmans 1972, Burnstock and Costa 1973) At least three alternatives might be considered as possible explanations to the present results (Fig. 4)

In paper I it was shown that the excitatory effect on the ICS following splanchnic stimulation was blocked by the ganglionic blocking agent chlorisondamine (2 mg/kg b.w.) while the contraction could be elicited again when the periarterial nerves, which are considered to be constituted mainly by postganglionic neurons of the splanchnic nerves, were stimulated (Fig. 5 panel A, B and C) This might imply a direct effect of the adrenergic nerves *via* excitatory α-receptors on the sphincteric muscles (B in Fig. 4) as it was shown in I and II that the contraction of the ICS following periarterial nerve stimulation was blocked by guanethidine (Fig. 5 panel E) and phenoxybenzamine but not by propranolol. There is anatomical support for this assumption as Costa and Gabella (1971) and Silva *et al* (1971) have shown that adrenergic nerves not only terminate in the intramural plexuses as suggested by Norberg (1964) and Jacobowitz (1965) but are also present within the circular muscle layer of all regions of the subdiaphragmatic digestive tract.

However as previously mentioned, Kosterlitz and Watt (1965) suggested that the α-receptors within the intestine are located at the intramural cholinergic ganglion cells. This alternative explanation would imply that postganglionic adrenergic fibres act on the intramural cholinergic ganglion cells within the ICS, as shown in A in Fig. 4 The effect of adrenergic nerve stimulation would then be an increased liberation of acetylcholine at the postganglionic nerve endings as it was indicated in III that cholinergic nerve stimulation elicited a contraction of the ICS. However one must then postulate that such adrenergic synapses would be resistant to chlorisondamine (Fig. 5 panel C)

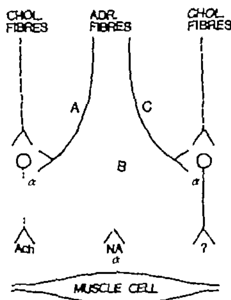


Fig. 4 Three different alternatives for the adrenergic innervation of the smooth muscle cells in the ICS. For dicussion see text.
A. Nervous termination on intramural cholinergic ganglions.
B. Nervous termination on sphincteric smooth muscle cells.
C. Nervous termination on intramural ganglion cells with unknown peripheral transmitter

A final common pathway for the cholinergic and adrenergic nervous systems was discussed by Christensen and Daniel (1968). They found that cholinergic as well as adrenergic drugs elicited a contraction of the lower oesophageal sphincter of the cat *in vitro* and that the response to both kind of drugs was blocked by atropine. The excitatory motor response in the ICS following adrenergic nerve stimulation was not blocked by a low dose of atropine and only transiently by a larger dose (Fig. 5 panel D), so that this explanation is unlikely

A third possible explanation for the adrenergic excitatory motor response in the *ICS might be that the adrenergic nerves exert influence via* α-receptors on non cholinergic, non adrenergic nerve fibres within the ICS (C in Fig. 4) Although such fibres are considered generally to be inhibitory (Burnstock 1972), excitatory fibres of this kind have been shown to supply the small intestine of the cat (Day and Warren 1968) Although such an explanation for the excitatory response in the *sphincter following adrenergic nerve stimulation cannot be excluded from the* present experiments, far too little is known at present about such non-cholinergic, non adrenergic excitatory fibres to postulate this mechanism as the cause of the con traction of the sphincter

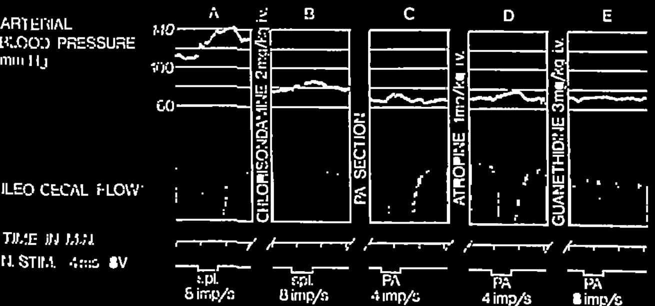


Fig. 5 Effect of splanchnic nerve stimulation before and after chlorisondamine (Panel A and B). Effect of periarterial nerve stimulation after chlorisondamine, atropine and guanethidine respectively (Panel C, D and E). For details, see text.

Thus, in the light of present knowledge, the suggestion of a direct effect of the adrenergic nerves *via* excitatory α-receptors on the sphincteric muscle seems most reasonable. Such an assumption might imply at least a quantitative difference in the peripheral innervation between the ICS and other gastrointestinal smooth muscles where the adrenergic nerves are considered to exert their effect mainly by an inhibitory action on the intramural pro- and postganglionic cholinergic transmission (Kewenter 1965 Jansson and Martinson 1966, Hultén 1969 Paton and Vizi 1969 Beani *et al* 1969) Such quantitative difference in the location of the adrenergic terminals in the intestine and the ICS might possibly have a relation to the difference in function between the α-receptors in these two parts of the gastrointestinal tract. An alternative explanation is a difference in responsiveness to the transmitter in the smooth muscle cells from different parts of the intestine (e.g. Gazet and Jarrett 1964)

Reflex activation of the ICS by intestinal distension (IV)

Distension of a small or large intestinal loop isolated from the adjacent gut but with the mesenteric vascular and nervous supply intact, decreased or abolished the transsphincteric flow As there was a concomitant inhibition of intestinal motor activity it was concluded that the reduced transsphincteric flow was due to pure sphincter contraction.

Contraction of the ICS following large intestinal distension is in agreement with the physiological characteristics of a gastrointestinal sphincter as defined by e.g. Cohen *et al* (1968) and Fisher and Cohen (1973) while the contraction of the sphincter elicited by distension of the small intestine is contradictory to their definition in which the tone of the sphincter is considered to be decreased by pre-sphincteric stimulation. Such relaxant responses have been recorded in the ICS of the dog and man following ileal distension (Kelley Jr *et al* 1966, Cohen *et al* 1968) Excitatory responses in the ICS following small intestinal distension, as in the present study were found by Hinrichsen and Ivy (1931) in dogs while Chang and Hsu (1942), also in dogs, obtained contraction when the tone of the sphincter was low and relaxation when the tone was high. In the present study no relaxation of the sphincter could be recorded even when the tone of the sphincter had been raised by either adrenergic nerve stimulation or distension of another intestinal loop.

Nerve section and spinal anesthesia. As the vessels of the suprarenal glands were ligated and the continuity between the intestinal loops and the ileo-cecal region was broken an extrinsic nervous pathway for the reflex was concluded. The pathway for the excitatory intestino-ileo-cecal sphincteric reflex elicited by small intestinal distension was further studied by nerve section and spinal anesthesia. The cranial and sacral parasympathetic outflow could be excluded as mediators of the reflex as this was not affected by section of the vagal and/or pelvic nerves.

Section of the major splanchnic and lumbar colonic nerves, but not one or the other completely or almost completely abolished the response and these nerves were therefore considered as the major pathways for the reflex. This is not in agreement with the findings of Hinrichsen and Ivy (1931) and Chang and Hsu (1942) The former authors considered the reflex to be dependent upon both the vagal and splanchnic nerves, while the latter authors concluded that the splanchnic nerves were the only pathways for the reflex.

Spinal anesthesia totally abolished the reflex. Small intestinal distension is known to elicit inhibition in other parts of the gastrointestinal canal (Johansson and Langston 1964 Jansson and Martinson 1966, Hultén 1969). Johansson and Langston (1964) could clearly show that the intestino-intestinal inhibitory reflex was spinal and not mediated *via* a synaptic mechanism in the mesenteric ganglia (Kuntz and van Buskirk 1941 Kuntz and Saccomanno 1944). It could further be shown that the reflex was under supraspinal control (Johansson *et al* 1965 and 1968) A similar spinal pathway for the intestino-ileo-cecal sphincter reflex is also indicated from the present study but whether it is under supraspinal control or not was not investigated.

Autonomic blockade As the reflex contraction of the sphincter was not blocked by atropine or propranolol but was blocked by guanethidine and phenoxybenzamine it was concluded that the reflex was mediated *via* an excitatory adrenergic α-receptor mechanism just as the response following sympathetic nerve stimulation.

Thus, intestinal distension elicits a contraction of the ICS. This excitatory intestino-ileo-cecal sphincteric reflex is proposed to be a sympathetic spinal reflex mediated *via* an α-adrenergic mechanism.

FUNCTIONAL CONSIDERATIONS

The ICS might have two functions, to prevent regurgitation of material from the cecum into the small bowel and to delay the passage of the ileal contents into the cecum (Alvarez 1948). In the present study almost exclusively contractory effects on the sphincter were obtained both upon nerve stimulation and intestinal distension thus supporting the function of the ICS as being to keep the ileal and colonic contents apart.

However the opening mechanism of the sphincter is not clear. Several factors may contribute to the relaxation of the sphincter e.g. decreased tonic activity in the adrenergic and cholinergic nerves to the sphincter. Activation of possible inhibitory β-receptors in the ICS and/or reflex relaxation of the sphincter similar to that described by Kelley Jr *et al.* (1966) and Cohen *et al* (1968) are other alternatives as it cannot be excluded that the continuity of the intestine might be of importance in mediating a reflex inhibition of the ICS. An additional explanation for decreased sphincter tone might be the influence of gastrointestinal hormones which may take part in the regulation of intestinal tone and motility (Makhlouf 1974). Recently gastrin, both endogenous and exogenous, has been shown to decrease the tone of the ICS in man (Castell *et al.* 1970). The effect of the pelvic nerves on the ICS is not known. Inhibitory fibres within these nerves to the sphincter cannot be excluded.

SUMMARY

The influence on the ileo-cecal sphincter (ICS) of electrical efferent stimulation of the splanchnic, lumbar colonic and vagal nerves and of intestinal distension was studied in anesthetized cats. A new flow recording technique was designed. Reponses in the ICS to stimulation were recorded as changes in the transsphincteric flow The response in the ICS was compared to the responses in the intestine proximal and distal to the sphincter Sympathomimetic amines and autonomic blocking drugs were used in order to elucidate the autonomic mechanism by which the responses to stimulation were mediated

On the basis of the observations made the following conclusions were drawn

1 Both the splanchnic and the lumbar colonic nerves exert a contractile influence on the ICS. The effect is mediated *via* excitatory adrenergic α-receptors.

2. The ICS contains inhibitory adrenergic β receptors.

3. Activation of the vagal nerves causes a decrease of the transsphincteric flow which is at least partly due to a direct nervous effect on the sphincteric muscle. A cholinergic mechanism is suggested.

4. Intestinal distension elicits contraction of the ICS This excitatory intestino-ileo-cecal sphincteric reflex is a spinal reflex and mediated *via* adrenergic fibres within the sympathetic nervous system acting on α-receptors.

ACKNOWLEDGEMENTS

I wish to express my sincere gratitude to my friend and collaborator docent Jan Kewenter for his never fading interest and support throughout this work. I am also very grateful to professor Torsten Sundin and docent Olof Jonsson for their valuable help.

For most skilful technical assistance I thank Harriet Andersson. Thanks are also due to Agneta Sirén for excellent typing of the manuscripts.

I am indebted to professors Yngve Edlund, Lars-Erik Gelin and Nils G Kock for their encouragement and support given to this study

Financial aid was received from Göteborgs Läkaresällskap and the Swedish Medical Research Council (project No B75-17X 577 11A)

REFERENCES

AHLQUIST R. P Agents which block adrenergic β-receptors. *Ann. Rev Pharmacol* 1968. *8* 259—272.

AHLQUIST R. P and B. LEVY Adrenergic receptive mechanism of canine ileum. *J Pharmacol. exp. Ther* 1959 *127* 146—149

ALVAREZ, W C., The ileocecal sphincter In *An Introduction to gastroenterology* Hoeber New York, 1948.

BASS, D. D., T J USTACH and M. M. SCHUSTER, In vitro pharmacologic differentiation of sphincteric and non-sphincteric muscle. *John Hopkins Med. J* 1970. *127* 185—191.

BEANI, L., C. BIANCHI and A. CREMA, The effect of catecholamines and sympathetic stimulation on the release of acethylcholine from the guinea pig colon. *Brit. J Pharmacol.* 1969 *36* 1—17

BOYD H., G. BURNSTOCK, G. CAMPBELL, A. JOWETT J O'SHEA and M. WOOD, The cholinergic blocking action of adrenergic blocking agents in the pharmacological analysis of autonomic innervation. *Brit J Pharmacol* 1963. *20* 418—435.

BRODY T M. and J DIAMOND Blockade of the biochemical correlates of contraction and relaxation in uterine and intestinal smooth muscle. *Ann. N Y Acad. Sci* 1967 *139* 772—780.

BUCKNELL, A. and B. WHITNEY A preliminary investigation of the pharmacology of the human isolated taenia coli preparation. *Brit J Pharmacol.* 1964 *23* 164—175.

BURNSTOCK, G., Purinergic nerves. *Pharmacol Rev* 1972. *24* 509—581.

BURNSTOCK, G. and M. COSTA, Inhibitory innervation of the gut. *Gastroenterology* 1973. *64* 141—143.

CASTELL, D. O S. COHEN and L. D. HARRIS, Response of human ileocecal sphincter to gastrin. *Amer J Physiol.* 1970. *219* 712—715

CHANG, P Y and F Y HSU The localization of the intestinal inhibitory reflex arc. *Quart J exp. Physiol* 1942. *31* 311—318.

CHRISTENSEN, J The adrenergic nerves and gastrointestinal smooth muscle function. *Gastroenterology* 1968. *55* 135—138.

CHRISTENSEN, J The controls of gastrointestinal movements. Some old and new views. *New Engl. J Med.* 1971 *285* 85—98.

CHRISTENSEN, J and E. E. DANIEL, Effects of some autonomic drugs on circular esophageal smooth muscle. *J Pharmacol exp. Ther* 1968. *159* 243—249

CLARK, C. G. and J R. VANE, The cardiac sphincter in the cat. *Gut* 1961 *2* 252—262.

CODE, C. F and J F SCHLEGEL, Motor action of the esophagus and its sphincters. In *Handbook of Physiology* Sect. 6. Vol. IV Amer Physiol. Soc. Washington 1968. 1821—1839

COSTA, M. and G. GABELLA, Adrenergic innervation of the alimentary canal. *Z. Zellforsch.* 1971 *122.* 357—377

COHEN, S., L. D. HARRIS and R. LEVITAN Manometric characteristics of the human ileocecal junctional zone. *Gastroenterology* 1968. *54* 72—75.

DALE, H. H., On some physiological actions of ergot. *J Physiol* (Lond.) 1906. *34* 163—206.

DAY M. D. and P R. WARREN A pharmacological analysis of the responses to transmural stimulation in isolated intestinal preparations. *Br J Pharmacol Chemother* 1968. *32* 227—240.

DIDIO L. J A. and M. C. ANDERSSON *The "Sphincters of the Digestive System*. Williams and Wilkins 1968. 157—196.

ELLIOTT T R., On the innervation of the ileo-colic sphincter *J Physiol* (Lond.) 1904. *31* 157—168.

FISHER, R. and S. COHEN Physiological characteristics of the human pyloric sphincter *Gastroenterology* 1973 *64* 67—75

FLEISCHNER, F G and C. BERNSTEIN Roentgen-anatomical studies of the normal ileocecal valve. *Radiology* 1950. *54* 43—58.

FRIEDELL, M. T and E. G. WAKEFIELD The ileocecal valve of man. *Proceedings of the staff meetings of the Mayo Clinic* 1941 *16* 705—707

FURCHGOTT R. F Receptors for sympathomimetic amines. In *Ciba Foundation Symposium, Adrenergic Mechanisms*. Churchill, London 1960 246—252.

GADDUM J H., An outflow recorder. *J Physiol* (Lond.) 1929 *67* 16—17

GARRY R. C., The nervous control of the caudal region of the large bowel in the cat. *J Physiol* (Lond.) 1933. *77* 422—431

GAZET J-C. and R. J JARRETT The ileocaeco-colic sphincter Studies in vitro in man, monkey cat and dog. *Brit. J Surg* 1964. *51* 368—370.

GAZET J-C., The surgical significance of the ileo-caecal junction. *Ann. Roy Coll Surg Engl* 1968. *43* 19—38.

HAMMER, G Untersuchungen über die Funktion der Valvula Bauhini. *Deutsch. Arch. klin. Med.* 1927 *157* 1—19

HIGGS, B R. G SHORTER and F H. ELLIS Jr A study of the anatomy of the human esophagus with special reference to the gastroesophageal sphincter *J Surg Res.* 1965 *5* 503—507

HINRICHSEN J and A. C. IVY Studies on the ileo-cecal sphincter of the dog. *Amer J Physiol* 1931 *96* 494—507

HROMADA, G Zur Insuffizienz der Valvula Bauhini. *Arch. Klin. Chir* 1921 *117* 784—818.

HULTEN L., Extrinsic nervous control of colonic motility and blood flow *Acta physiol scand*. 1969 Suppl. 335.

INNES, J R. and M. NICKERSON Drugs acting on postganglionic adrenergic nerve endings and structures innervated by them (Sympathomimetic drugs). In *The Pharmacological Basis of Therapeutics*. Goodman and Gilman. 1968. 3rd ed. 427—520.

INNES, J R. and M. NICKERSON Drugs inhibiting the action of acetylcholine on structures innervated by postganglionic parasympathetic nerves. In *The Pharmacological Basis of Therapeutics*. Goodman and Gilman. 1968. 3rd ed. 521—545

JACOBOWITZ, D Histochemical studies of the autonomic innervation of the gut. *J Pharmacol exp. Ther* 1965 *149* 358—364

JANSSON G and J MARTINSON Studies on the ganglionic site of action of sympathetic outflow to the stomach. *Acta physiol scand*. 1966. *68*. 184—192.

JARRETT R. J and J-C. GAZET Studies *in vivo* of the ileocaeco-colic sphincter in the cat and dog. *Gut* 1966 *7* 271—275

JOHANSSON B. and J B. LANGSTON Reflex influence of mesenteric afferents on renal, intestinal and muscle blood flow and on intestinal motility *Acta physiol scand.* 1964. *61* 400—412.

JOHANSSON B., O JONSSON and B. LJUNG Supraspinal control of the intestino-intestinal inhibitory reflex. *Acta physiol scand.* 1965.*63* 442—449

JOHANSSON B., O JONSSON and B. LJUNG Tonic supraspinal mechanisms influencing the intestino-intestinal inhibitory reflex. *Acta physiol scand.* 1968, *72*, 200—204
KALSER, M H., J L A. ROTH, H. TUMEN and T A. JOHNSON Relation of small bowel resection to nutrition in man. *Gastroenterology* 1960, *38* 605—615.
KATSCH, G., Der menschliche Darm bei pharmacologischer Beeinflussung seiner Innervation. Insufficienz der Sphincter Ileo-colicus. *Fortschr Roentgenstr* 1913 *21* 177—179
KELLEY M. L. Jr., E. A. GORDON and J A. DEWEESE, Pressure studies of the ileocolonic junctional zone of dogs. *Amer J Physiol* 1965, *209* 333—339
KELLEY M. L. Jr E. A. GORDON and J A. DEWEESE, Pressure responses of canine ileocolonic junctional zone to intestinal distention. *Amer J Physiol* 1966, *211* 614—618.
KEWENTER, J *The vagal control of the jejunal and ileal motility and blood flow* *Acta physiol. scand.* 1965, *65*, Suppl. 251
KOCK, N G., An experimental analysis of mechanisms engaged in reflex inhibition of intestinal motility *Acta physiol. scand.* 1959 *47* Suppl. 164.
KOCK, N G., L. HULTÉN and L. LEANDOER, A study of the motility in different parts of the human colon. *Scand. J Gastroent* 1968, *3* 163—169
KOSTERLITZ, H. W and A. J WATT Adrenergic receptors in the guinea-pig ileum. *J Physiol* (Lond.) 1965 *177* 11—12P
KUNTZ, A. and C. VAN BUSKIRK, Reflex inhibition of bile flow and intestinal motility mediated through decentralized celiac plexus. *Proc Soc. exp. Biol. N Y* 1941 *46*. 519—523.
KUNTZ, A. and G SACCOMANNO Reflex inhibition of intestinal motility mediated through decentralized prevertebral ganglia. *J Neurophysiol* 1944 *7* 163—170.
LENZ, H., Der Öffnungs- und Verschlussmechanismus der Valvula Bauhini. *Fortschr Roentgenstr* 1964 *101* 154—165
LEVITT M. N., H. H. DEDO and J H. OGURA, The cricopharyngeus muscle, an electromyographic study in the dog. *Laryngoscope* 1965, *75* 122—136.
LUDUENA, F P and M. J BRANIN Adrenolytic activity of atropine, (+)-hyoscyamine, atroscine, homatropine and related compounds. *J Pharmacent. Sci* 1966, *55* 280—284
MAKHLOUF G. M., The neuroendocrine design of the gut. *Gastroenterology* 1974, *67* 159—184.
MARRAZZI, A. S., Electrical studies on the pharmacology of autonomic synapses. I. The action of parasympathomimetic drugs on sympathetic ganglia. *J Pharmacol. exp. Ther* 1939 *65* 18—35.
MARTIN J S., D. L. INNES and M. F TANSY A demonstration of vagal adrenergic vascular and motor influences in the small intestine of the dog. *Surg. Gyn. Obstet.* 1974, *138* 6—12.
MARTINSON, J Vagal relaxation of the stomach. Experimental reinvestigation *of the concept* of the transmission mechanism. *Acta physiol scand.* 1965 a, *64* 453—462.
MILLER, R. E., Complete reflux small bowel examination. *Radiology* 1965, *84* 457—463.
MUNRO, A. F The effect of adrenaline on the guinea pig intestine. *J Physiol* (Lond.) 1951 *112*, 84—94
NORBERG K.-A., Adrenergic innervation of the intestinal wall studied by fluorescence microscopy *Int. J Neuropharmacol* 1964, *3* 379—382.
PATON W D. M and E. S. VIZI, The inhibitory action of noradrenaline and adrenaline on acetylcholine output by guinea pig ileum longitudinal muscle strip. *Brit J Pharmacol* 1969 *35* 10—28

PERSSON C. G. A., Dual effects on the sphincter of Oddi and gallbladder induced by stimulation of the right great splanchnic nerve. *Acta physiol scand* 1973 87 334—343.

REDDY V A pharmacological analysis of adrenergic receptors in the isolated small intestine of the rabbit. Thesis for *M. S. Emory University* 1960.

RENDLEMAN D. F J. E. ANTHONY C. DAVIS Jr R. E. BUENGER, A. J BROOKS and E. J BEATTIE Jr Reflux pressure studies on the ileocecal valve of dogs and humans. *Surgery 1958 44* 640—643.

REYNOLDS, D G G. E. DEMAREE and M H. HEIFFER, An excitatory adrenergic alpha receptor mechanism of terminal guinea-pig ileum. *Proc Soc exp. Biol N Y* 1967 *125* 73—78.

ROSENBERG J C. and L. J. A. DIDIO, *In vivo* appearance and function of the termination of the ileum as observed directly through a cecostomy *Amer J Gastroenterol* 1969 *52*. 411—419.

SILVA, D.-G G ROSS and L. W OSBORNE, Adrenergic innervation of the ileum of the cat. *Amer J Physiol* 1971 *220* 347—352.

SINGLETON, A. O Jr D C. REDMOND and J E. MC MURRAY Ileocecal resection and small bowel transit and absorption. *Ann. Surg* 1964. *159* 690—694.

STAHLGREN L. H., G. UMANA, R. ROY and J DONNELLY A study of intestinal absorption in dogs following massiv small intestinal resection and insertion of an antiperistaltic segment. *Ann. Surg* 1962. *156* 483—492.

STAVNEY L. S., T KATO C. A. GRIFFITH, L. M NYHUS and H. N HARKINS, A physiological study of motility changes following selective gastric vagotomy *J Surg Res.* 1963. *3* 390—394

THOMAS, J E. and M V BALDWIN Pathways and mechanisms of regulation of gastric motility In *Handbook of Physiology* Sect. 6. Vol. IV Amer Physiol. Soc. Washington, 1968. 1937—1968.

TÖNNIS, W Die Funktion der Valvula ileocoecalis. *Pflüger Arch. ges. Physiol* 1924. *96* 477—482.

ULIN A. W W C. SHOEMAKER and J DEUTSCH, The ileocecal valve and papilla. *A M A Arch. Int Med.* 1956. *97* 409—420.

YOUMANS, W B., Do adrenergic nerves innervate smooth muscle in the intestinal wall? *Gastroenterology* 1972. 62. 1278—1279

PERSSON C. G A., Dual effects on the sphincter of Oddi and gallbladder induced by stimulation of the right great splanchnic nerve. *Acta physiol scand.* 1973 *87* 334—343.

REDDY V., A pharmacological analysis of adrenergic receptors in the isolated small intestine of the rabbit. Thesis for M S. Emory University 1960.

RENDLEMAN D. F J H. ANTHONY C. DAVIS Jr R. E. BUENGER A. J BROOKS and E. J BEATTIE Jr Reflux pressure studies on the ileocecal valve of dogs and humans. *Surgery* 1958 *44* 640—643.

REYNOLDS, D G G E. DEMAREE and M. H. HEIFFER, An excitatory adrenergic alpha receptor mechanism of terminal guinea pig ileum. *Proc. Soc exp. Biol N Y* 1967 *125* 73—78.

ROSENBERG J C. and L. J A. DIDIO *In vivo* appearance and function of the termination of the ileum as observed directly through a cecostomy *Amer J Gastroenterol.* 1969 *52* 411—419

SILVA, D-G G. ROSS and L. W OSBORNE, Adrenergic innervation of the ileum of the cat. *Amer J Physiol.* 1971 *220* 347—352.

SINGLETON A. O Jr., D C. REDMOND and J E. MC MURRAY Ileocecal resection and small bowel transit and absorption. *Ann. Surg* 1964 *159* 690—694.

STAHLGREN L. H., G UMANA, R. ROY and J DONNELLY A study of intestinal absorption in dogs following massiv small intestinal resection and insertion of an antiperistaltic segment. *Ann. Surg* 1962. *156* 483—492.

STAVNEY L. S., T. KATO C. A. GRIFFITH, L. M. NYHUS and H. N HARKINS, A physiological study of motility changes following selective gastric vagotomy *J Surg Res.* 1963. *3* 390—394.

THOMAS, J E. and M. V BALDWIN Pathways and mechanisms of regulation of gastric motility In *Handbook of Physiology* Sect. 6. Vol. IV Amer Physiol. Soc. Washington, 1968. 1937—1968.

TONNIS, W., Die Funktion der Valvula ileocoecalis. *Pflüger Arch. ges. Physiol.* 1924. *96.* 477—482.

ULIN A. W W C. SHOEMAKER and J DEUTSCH, The ileocecal valve and papilla. *A M A Arch. Int Med.* 1956. *97* 409—420.

YOUMANS, W B., Do adrenergic nerves innervate smooth muscle in the intestinal wall? *Gastroenterology* 1972. *62.* 1278—1279

KUNGSBACKA
ELANDERS BOKTRYCKERI AKTIEBOLAG
1975

ACTA PHYSIOLOGICA SCANDINAVICA

SUPPLEMENTUM 420

From the Department of Physiology Karolinska Institutet,
Stockholm 60, Sweden

and

the Department of Pharmacology University of Helsinki,
Helsinki 17 Finland

CONTRACTION AND RELAXATION OF THE RETRACTOR PENIS MUSCLE AND THE PENILE ARTERY OF THE BULL

A study of effects of drugs and transmural nerve stimulation on isolated smooth muscle strips

By

Erik Klinge
Department of Pharmacology
University of Helsinki
Helsinki

and

Nils O Sjöstrand
Department of Physiology
Karolinska Institutet
Stockholm

VAMMALA 1974

Vammala 1974
Vammalan Kirjapaino Oy
Finland

CONTENTS

	INTRODUCTION	5
CHAPTER I	SURVEY AND SCRUTINY OF EARLIER LITERATURE	7
	Historical Survey	7
	Summing Up and Critical Comments	13
CHAPTER II	BRIEF ANATOMICAL OUTLINE	17
CHAPTER III	MATERIAL AND METHODS	20
CHAPTER IV	RESULTS	23
	The Retractor Penis Muscle	23
	General properties and responses to nerve stimulation	23
	Pharmacological studies	32
	Agents blocking axonal conduction	32
	Sympathomimetic amines and adrenergic receptor blocking agents	33
	Adrenergic neuron blocking agents and other compounds influencing adrenergic mechanisms	35
	Parasympathomimetic and antimuscarinic agents	37
	Ganglionic stimulating and blocking agents	37
	Neuromuscular blocking agents	42
	Agents interfering with the synthesis, destruction or release of acetylcholine	42
	Histamine, 5-hydroxytryptamine and agents blocking their receptors	43
	Prostaglandins, prostaglandin antagonists and inhibitors of prostaglandin synthesis	44
	Amino acids	46
	Nucleosides and nucleotides	46
	Peptides	46
	Adrenal steroids	49
	Miscellaneous compounds	49
	The Penile Artery	50
	General properties and responses to nerve stimulation	50
	Pharmacological studies	54
	Agents blocking axonal conduction	54
	Sympathomimetic amines and adrenergic receptor blocking agents	55
	Adrenergic neuron blocking agents and ther compounds influencing adrenergic mechanisms	56

Parasympathomimetic and antimuscarinic agents 58
Ganglionic stimulating and blocking agents 58
Neuromuscular blocking agents 58
Agents interfering with the synthesis, destruction or release of acetylcholine 59
Histamine, 5-hydroxytryptamine and agents blocking their receptors 60
Prostaglandins, prostaglandin antagonists and inhibitors of prostaglandin synthesis 61
Amino acids 61
Nucleosides and nucleotides 61
Peptides 62
Miscellaneous compounds 63

CHAPTER V COMMENTS 65
General comments drug penetration and solvent effects 65
α-Adrenoceptor and adrenergic neuron blocking agents 65
β-Adrenoceptors 66
Some quaternary ammonium compounds 66
Local anaesthetics 66
Hemicholinium, botulinum toxin and morphine 67
Prostaglandins and prostaglandin receptors 67
Smooth muscle stimulants 68
Quinidine and ATP receptors 69

CHAPTER VI DISCUSSION 70
The smooth muscle of the retractor penis and the penile artery 70
Excitatory nerves and motor responses to field stimulation 70
Inhibitory nerves and relaxations induced by field stimulation 72
The rebound contraction 74
Acetylcholine and the role of possible cholinergic innervation 74
Posterior pituitary hormones 76
Some final remarks 78

SUMMARY 78

CONCLUSIONS 80

ACKNOWLEDGEMENTS 81

REFERENCES 83

INTRODUCTION

Erection of the mammalian penis is due to engorgement of the erectile tissue, i.e. the cavernous bodies of the organ. It is generally believed that the primary event in swelling of the erectile tissue is a profound dilation of the arteries forming the inflow channels to the cavernous bodies. This leads to a rapid filling of the erectile tissue because the »inflow resistance» of the tissue will be lower than the »outflow resistance». Cessation of erection is believed to be due to a restoration of the tone of the inflow vessels. Owing to this the »inflow resistance» of the cavernous bodies exceeds the »outflow resistance» and the erectile tissue is emptied.

In most subprimate mammals a second and often as important event in erection is a sudden relaxation of the retractor penis muscle. This predominantly smooth muscle is normally continuously contracted and keeps the relaxed penis withdrawn in the preputium under the skin, hereby protecting the glans penis from damage and contamination with the earth.

Thus, apparently a fundamental mechanism of erection is relaxation of smooth muscle cells, namely those of the arteries supplying the cavernous bodies and those of the retractor penis muscle. On the other hand, a principal mechanism maintaining the penis in the relaxed state is contraction of these smooth muscle cells.

The autonomic nervous pathways responsible for the erection as well as for the maintenance of the resting state of the penis were essentially mapped out during the 19th century by pioneers such as C. Eckhard, Ch. Lovén, W. H. Gaskell, M. François-Franck, J. N. Langley and H. K. Anderson. Their studies showed that the sacral parasympathetic outflow is the main nervous pathway for erection and the lumbar sympathetic outflow the pathway for penile vasoconstriction and contraction of the retractor penis muscle. According to our current concept of autonomic neurotransmission, the neurotransmission of erection would be cholinergic and the neurotransmission of penile vasoconstriction and contraction of the retractor penis muscle adrenergic. However hitherto published studies of this subject have not unanimously pointed to a cholinergic neuromuscular transmission of penile erection. Thus rather conflicting results have been obtained by different authors concerning the action of acetylcholine on the penile vessels and the retractor penis muscle. Further many authors have reported that the erection is resistant to atropine while some investigators have found that erection is suppressed by atropine.

This study deals with the effect of drugs, especially of neurotransmitters, and with the effect of nerve stimulation on isolated smooth muscle strips of the retractor penis muscle and the penile artery of the bull. Special attention has been paid to the question: Is the autonomic neurotransmission of erection cholinergic or not? Our hope was that a study of isolated tissues in a controllable environment might offer some further information on the autonomic

control of the tone of the smooth muscle cells ultimately responsible for penile erection in the mammalian male. In addition to own experiments we have tried to scrutinize the literature available in this field. The effect of adjacent striated muscles on the establishment and maintenance of penile erection is beyond the scope of the present study

CHAPTER I

SURVEY AND SCRUTINY OF EARLIER LITERATURE

At least four review articles have been written which include accounts of the innervation and the pharmacology of the penile vessels and the retractor penis muscle (Müller and Dahl 1912, Gruber 1933 Klinge 1969 a and Bell 1972). However these reviews do not go into details of this subject. Further the two recent articles are rather incomplete concerning work of not English speaking investigators. The survey presented here has deliberately been restricted to studies of the peripheral innervation of the above-mentioned target organs and to the effects of autonomic drugs on these organs.

Historical Survey

The great pioneer in the physiology of penile erection is undoubtedly Eckhard (1863, 1869). It is true that many earlier investigators, as e.g Müller (1835) and Kölliker (1852) had made several important observations and suggestions. Especially noteworthy is the idea of Kölliker that relaxation of the penile arteries, the smooth muscles of the cavernous bodies and the retractor penis constitutes a most essential condition for erection. Another noteworthy suggestion of Kölliker was that the tone of these smooth muscles was controlled by ganglia at the root of the penis and that the activity of these ganglia was enhanced by the sympathetic system and inhibited by the pelvic nerves. But it was Eckhard who showed that in the dog the main event in penile erection is dilation of the penile arteries. Eckhard (1863) was also the first to demonstrate that penile erection is produced by electric stimulation of the pelvic parasympathetic nerves, which he accordingly called the *nervi erigentes* He further found that section of the pudic nerves did not produce erection. Eckhard noted that during erection the blood flow in the penile veins is increased and not decreased. Hence it was Eckhard who created the modern concept that the erectile tissue is engorged because the inflow resistance of the cavernous bodies is lower than the outflow resistance. Since then the idea of Eckhard has been confirmed in many studies on dogs including the recent ones of Hart and Kitchell (1966) and Dorr and Brody (1967). This does not exclude that in several mammals events as relaxation of smooth muscle cells within the cavernous bodies, contraction of adjacent striated muscles and certain vasocompression play a role in penile erection (cf Chapter VI). Eckhard (1876) further found that in rabbits, but not in dogs, erection could also be produced by stimulation of the hypogastric nerve.

control of the tone of the smooth muscle cells ultimately responsible for penile erection in the mammalian male. In addition to own experiments we have tried to scrutinize the literature available in this field. The effect of adjacent striated muscles on the establishment and maintenance of penile erection is beyond the scope of the present study

the existence of a synaptic relay in the course of the nerve. Atropine, on the other hand, did not inhibit penile erection evoked by stimulation of the pelvic nerve. The effect of atropine was studied on rabbit which is, like man, devoid of the retractor penis muscle. Spina (1897) using the guinea-pig, also reported that atropine did not prevent penile erection.

Fletcher (1897) working with hedgehog and rat, reported that the excitatory supply (pudic nerve) and the inhibitory supply (pelvic nerve) formed a joint terminal meshwork in the retractor penis muscle. His description is indeed very close to the current concept of the autonomic ground plexus (Hillarp 1946, 1959). Fletcher found no nerve cells in the muscle and according to Fisher (1917) the same applies to the dog retractor penis.

De Zilwa (1901) made an *in vitro* study of the dog retractor penis. Like Sertoli (1883) he found that the smooth muscle showed spontaneous activity and that the tone increased in low but decreased in high temperatures. Electric stimulation of the muscles produced contraction when the muscle was relaxed, but often relaxation if it was in high tone. De Zilwa found that atropine usually relaxed the muscle. Muscarine, on the other hand, produced a slight contraction which could be abolished by atropine. Nicotine relaxed the muscle. De Zilwa further observed that *in vivo* the responses to pudic nerve stimulation were unaffected by atropine and curare.

Elliott (1905) found that in the dog the penile arteries and the retractor penis were contracted by adrenaline. He also found that the muscle showed denervation supersensitivity to this drug. Dale (1906) reported that ergot extracts by themselves caused a slight contraction of the dog retractor penis and abolished contractions induced by adrenaline or by stimulation of the pudic nerve. Thus the motor response of the retractor penis was included already in the two basic studies of adrenergic neurotransmission. In their study of effects of different sympathomimetic amines Barger and Dale (1910) noted only excitatory effects of these compounds on the dog and goat retractor penis but never inhibitory effects despite treatment with ergotoxine

Fröhlich and Loewi (1908) reported that the effect of pelvic nerve stimulation on the dog retractor penis could be paralyzed with very high concentrations of nitrite.

An early electrophysiological study was performed by Brücke (1910) who found that the contracted dog retractor penis showed rythmic electrical waves. These waves increased in frequency upon stimulation of the pudic nerve and were abolished by pelvic nerve stimulation (Brücke and Oinuma 1910).

In another of his classic papers Dale (1914) reported that *in vivo* acetyl choline relaxed the retractor penis muscle but *in vitro* the ester had no effect on the muscle According to Dale the *in vivo* effect was probably due to the drop in blood pressure caused by acetylcholine and not to a direct effect of acetylcholine on the retractor penis muscle since prolonged vagal stimulation also produced a relaxation of the muscle.

Bottazzi (1916) reported that *in vitro* the retractor penis of dog was contracted by histamine tyramine pilocarpine, muscarine and also by atropin but relaxed by papaverine. Edmunds (1920) confirmed that adrenaline contracted the dog retractor penis *in vitro* This contraction was bolished and even reversed by ergotoxine. The muscle was contracted by pilocarpine This contraction was abolished by atropine but not by ergotoxine. Atropine by itself caused a slight relaxation. Physostigmine produced a contraction, which was counteracted by atropine. In contrast to De Zilwas findings Edmunds

found that nicotine contracted the muscle This effect was also seen after ergotoxine and was therefore assumed to be a direct smooth muscle effect. Edmunds further reported that morphine usually contracted the muscle. Edmunds' paper is the last of the papers in this field published before the concept of chemical neurotransmission was conclusively established. In the following this concept is accepted by the authors and their considerations are influenced thereby

Henderson and Roepke (1933) using penile perfusion in dogs, found that physostigmine enhanced the effect of pelvic nerve stimulation on venous flow while atropine had almost no effect. Arterial injections of acetylcholine also increased the flow This effect was abolished by atropine. Henderson and Roepke found no certain amounts of acetylcholine in the perfusion fluid. They concluded that they could not present direct evidence for acetylcholine as neurotransmitter of erection, though the effect of eserine suggested that this was the case.

Bacq (1935) found that in dog eserine greatly enhanced the penile engorgement evoked by pelvic nerve stimulation and that atropine strongly counter acted this engorgement. The vasoconstrictor effect of pudic nerve stimulation was enhanced by cocaine. In agreement with the findings of François-Franck (1895) the sympathetic nerves were found to also carry dilator fibres the effect of which was enhanced by eserine and suppressed by atropine. Bacq stated that the neurotransmission of erection was cholinergic although he was, like Henderson and Roepke, unable to demonstrate any acetylcholine in the penile perfusate. He further explained the lack of effect of atropine found by so many other investigators to have been due to the circumstance that they had not worked with eserinized animals.

Oppenheimer (1938) studied the cat retractor penis *in vivo* He found that the frequency response curve to sympathetic stimulation as well as the dose-response curve to adrenaline were hyperbolic. Both types of response were enhanced by cocaine. The inhibitory frequency response curve to parasympathetic stimulation was also hyperbolic. He found that acetylcholine duplicated the effect of parasympathetic stimulation. The effect of acetylcholine was enhanced by eserine and abolished by atropine. However atropine did not abolish the effect of parasympathetic stimulation. Oppenheimer stated that the transmitter causing relaxation of the retractor penis was acetylcholine. The resistance to atropine of the parasympathetic response was explained by the concept of Dale and Gaddum (1930) i e. the muscle cell membranes are in such close proximity to the nerve terminals that their receptors are almost inaccessible to the blocking agent. This theory of Dale and Gaddum called the proximity theory» by Ambache (1955) has since then often been adopted in explaining the resistance to atropine of penile erection. Using cats Semans and Langworthy (1938) partially repeated and confirmed the experiments of Langley and Anderson (1895) concerning the origin of the motor and inhibitory nervous supply of the genital organs.

Luduena (1939) reported that ergotoxine, yohimbine and a piperidine derivative inhibited in the isolated dog retractor penis the response to adrenaline. Further studies (Luduena 1940) showed that cocaine enhanced the excitatory effect of adrenaline, epinine and synephrine, while it reduced that of tyramine and phenylethylamine Luduena and coworkers (1949) also found that isoprenaline relaxed the dog retractor penis.

The presence of acetylcholinesterase in the penile erectile bodies of rat and guinea pig was demonstrated by Grieten and Gerebtzoff in 1957 Moderate

doses of atropine have been reported not to impair penile erection in bull (Signoret 1962) rabbit and boar (Dziuk and Norton 1962, Dziuk and Mann 1963) while higher doses may cause insufficient erection in rabbit (Dziuk and Norton 1962) In the study of Baker *et al.* (1964) pilocarpine did not affect the bull's ability to erect the penis.

Orlov (1962, 1963 a and b) studied the autonomic neurotransmission of the dog retractor penis with microelectrodes. He found that the muscle exhibited spontaneous spike activity and also slow waves upon which bursts of spikes could be superimposed. Stimulation of the pudic nerve produced excitatory junction potentials (EJP:s) which showed summation and elicited spikes. Adrenaline depolarized the muscle and elicited spikes. Spontaneous miniature EJP:s were also seen. These were impaired by sympathetic denervation or reserpine treatment. Repetitive postganglionic» stimulation of the pelvic nerve produced an inhibitory junction potential» (IJP) and suppressed spontaneous spiking The IJP:s were inhibited by d tubocurarine and facilitated by physostigmine. Atropine reduced the IJP:s in 85 % of the experiments. High concentrations of acetylcholine hyperpolarized the cell membrane and relaxed the muscle. Orlov concluded that the IJP was due to acetylcholine released from the parasympathetic nerves.

Gushchin (1965) studied the effect of adrenaline and acetylcholine on the isolated dog retractor penis. Low concentrations of adrenaline contracted the muscle. These contractions were blocked by dihydroergotamine. Acetylcholine contracted the muscle and these contractions were inhibited by atropine. However when repeated doses of acetylcholine were given the contractile responses became smaller and eventually the response was reversed, i.e. when the acetylcholine concentration had increased the response was inhibition of the muscle tone. Dihydroergotamine had no effect on the acetylcholine contractions. Gushchin assumed that the effect of acetylcholine was dependent on the concentration of the drug and the state of contraction of the muscle. He postulated that parasympathetic nerve stimulation produced very high concentrations of acetylcholine at the neuromuscular junctions and hence the effect of nerve stimulation was relaxation of the muscle. The experiments did not support the idea of a cholinergic release of the adrenergic transmitter

In a short communication Goldenberg (1965) reported that injected nicotine produced atropine-fast relaxations in the cat retractor penis, which were abolished by hexamethonium. He believed that the effect of nicotine was due to stimulation of parasympathetic nerves but that the liberated transmitter was not acetylcholine.

Luduena and Grigas (1966) made a pharmacological study of the isolated field stimulated dog retractor penis. The response of the untreated muscle was contraction. This was blocked by phentolamine piperoxan bretylium, guanethidine and local anaesthetics. Bretylium and guanethidine also produced a gradually developing contraction and then an inhibitory response to nerve stimulation was uncovered. This inhibitory response was not abolished by atropine or scopolamine nor was it enhanced by physostigmine. It was not affected by hemicholinium, hexamethonium or dichloroisoproterenol (DCI) but blocked by propoxycaine Acetylcholine contracted the muscle and the contraction was abolished by atropine Histamine 5 hydroxytryptamine and nicotine contracted the muscle. The effect of nicotine was abolished by hexamethonium. Luduena and Grigas concluded that field stimulation activated excitatory postganglionic sympathetic adrenergic fibres and inhibitory postganglionic

parasympathetic fibres but that the neurotransmitter of the latter fibres was not acetylcholine. They further found no evidence for a cholinergic link in the adrenergic neurotransmission.

Armitage and Burn (1967) found that in scopolamine-treated dogs acetylcholine contracted the retractor penis muscle. After section of the sympathetic chain this effect was abolished, but then acetylcholine reduced the contractions brought about by injected noradrenaline They found that eserine enhanced the response to sympathetic stimulation and took these results as support for the theory of a cholinergic link in the adrenergic transmission.

Dorr and Brody (1967) studied the haemodynamics of erection in dogs. They found that stimulation of the pelvic nerves produced a decrease in the arterial resistance of the perfused penis. The effect of pelvic nerve stimulation was abolished by hexamethonium and partly reduced but not abolished, by atropine. However in their study acetylcholine produced no decrease in arterial resistance. No sign of erection was obtained by intra-arterial application of histamine, 5-hydroxytryptamine, brakykinin, DMPP prostaglandin A_1 noradrenaline, or nitroglycerine Dorr and Brody found it unlikely that acetylcholine would be directly responsible for the effect of pelvic nerve stimulation but could possibly release a potent vasodilator agent. They considered it likely that the effect of atropine could be due to action on the ganglionic synapse apparently present in the neurogenic pathway

Hukovic and Bubic (1967) studied the isolated cavernous bodies of the rabbit. The bodies were relaxed by acetylcholine as well as by stimulation of the pelvic nerve. The response to pelvic nerve stimulation was partly reduced by atropine. It was enhanced by physostigmine and hexamethonium. Stimulation of the sympathetic nerves produced contraction this also was diminished by atropine. Noradrenaline and histamine contracted the cavernous bodies. Hukovic and Bubic stressed the importance of cholinergic nerves in the physiological regulation of blood vessel dilation. Working with rabbits Thielen and coworkers (1969) arrived at the same conclusion as François-Franck (1895) and Bacq (1935) i.e that also the hypogastric nerve carries erectile fibres although the pelvic nerves constitute the main pathway of erection.

Penttilä and Vartiainen (1964) measured the content of acetylcholine, dopamine, noradrenaline and adrenaline in the cavernous bodies of rabbit and bull. All amines except dopamine were found in rabbit material. The corpus cavernosum penis of the bull lacked acetylcholine and had a very low content of noradrenaline and adrenaline, but like many other tissues from ruminants it contained dopamine Further studies revealed that the erectile tissues of bull had a very low acetylcholinesterase activity compared with rabbit erectile tissues (Penttilä 1966). Histochemical studies showed that the corpus cavernosum penis of bull lacked adrenergic and cholinesterase-positive nerve terminals, while such terminals were abundant in the erectile tissues of rabbit (Klinge and Penttilä 1969) However adrenergic fibres and slightly acetylcholinesterase-positive fibres have been demonstrated in the penile artery of the bull prior to its entrance into the cavernous bodies (Klinge 1969 b, Klinge and Pohto 1971). Adrenergic nerve terminals have also been observed in the penile vessels and the smooth muscles of the cavernous bodies of cats and macaques (Baumgarten, Falck and Lange 1969) Perfusion studies (Penttilä 1966, Penttilä and Klinge 1966) of bovine erectile tissue showed that adrenaline and noradrenaline increased the inflow resistance. After phentolamine the effect of adrenaline was reversed. Isoprenaline decreased the resistance and this effect was abolished by DCI. Acetylcholine had usually no effect on the resistance, but if

the perfusion pressure was high it decreased the resistance and increased the venous outflow This effect was blocked by atropine.

Klinge (1970 a) found a high content of noradrenaline in the retractor penis of the bull. The muscle was further reported to contain a relatively large amount of acetylcholine and to exhibit acetylcholinesterase activity (Klinge 1970 b). Histochemical studies revealed an abundance of adrenergic fibres and also acetylcholinesterase-positive fibres (Klinge, Pohto and Solatunturi 1970). There was some distributional correspondence between adrenergic fibres and acetylcholinesterase-positive fibres. Bell and McLean (1970) demonstrated adrenergic fibres along the whole length of the smooth muscle of the dog retractor penis. Acetylcholinesterase-positive nerve terminals were, however almost lacking in the posterior half of the smooth muscle but not in the anterior half. Bell and McLean concluded that the smooth muscle of the dog retractor penis might receive innervation from separate adrenergic and cholinergic fibres. Appreciable levels of acetylcholinesterase did not appear to be associated with adrenergic axons in the dog.

Klinge (1970 c) studied the responses to drugs of the isolated bull retractor penis muscle. It was vigorously contracted by low concentrations of adrenaline and noradrenaline and this effect was abolished by phenoxybenzamine and reversed if the muscle was contracted by bradykinin. The muscle strips were also contracted by histamine and 5-hydroxytryptamine. Acetylcholine in high concentrations irregularly contracted the muscle. Also propionylcholine, butyrylcholine, methacholine, carbamylcholine, bethanechol and nicotine produced contraction when high concentrations were applied. The muscle was further contracted by very low concentrations of bradykinin (Klinge 1969 c) On the base of the lack of relaxing effect of acetylcholine Klinge (1970 b and c, Klinge and Pohto 1971) put forward the hypothesis that the effectors of erection, i.e the smooth muscle of the penile arteries and the retractor penis, might be supplied by one single type of efferent fibres, i.e. sympathetic adrenergic fibres, and that erection were due to interruption of the sympathetic tone produced by discharges in cholinergic parasympathetic fibres synapsing on the adrenergic neurons. In guinea-pigs treated with reserpine the penis was filled with blood and slightly swollen although there was no actual erection of the organ (Klinge 197?)

In a recent report Luduena and Grigas (1972) studied the effect of various drugs on the dog retractor penis *in vitro* The muscle was contracted by histamine 5-hydroxytryptamine, adenosine triphosphate (ATP) and prosta glandin $F_{2\alpha}$ while γ-aminobutyric acid (GABA) was without effect. But low concentrations of prostaglandin E_1 relaxed the muscle. In referring to their previous study (1966) Luduena and Grigas considered PGE_1 to be the most likely candidate for the role of the parasympathetic transmitter in the dog retractor penis.

Summing Up and Critical Comments

The excitatory innervation of the penile vessels and the retractor penis muscle gives us little problem. It derives from the lumbar sympathetic out flow Most fibres emanate from the sacral ganglia of the sympathetic chain and reach the target organs via the pudic nerve Some fibres seem to run in the hypogastric nerve. A few fibres may follow the pelvic parasympathetic nerves. The excitatory neurotransmitter of the sympathetic fibres is

apparently noradrenaline. At least no study presents any doubt in this respect. However here as in other organs there exists a dispute concerning the possibility of a cholinergic link in the adrenergic neurotransmission (Burn and Rand 1965 Burn 1967).

If the excitatory innervation appears to be clarified, this hardly can be said of the inhibitory innervation of the penile vessels and the retractor penis muscle. All investigators agree that the sacral parasympathetic nerves carry inhibitory fibres to the target organs in question. But some authors have also found vasodilator fibres in the sympathetic nerves. This could be the case since erection has been reported to occur in dogs (Müller 1902) and cats (Root and Bard 1947) with severed pelvic nerves when the males mount oestrous females. This points to possible alternative neuronal mechanisms of erection than discharges in the pelvic nerves. When we consider the inhibitory neurotransmission of erection the data available in the literature are indeed both ambiguous and contradictory Naturally this also applies to the opinions presented by different investigators working in this field. The main question at present is Is the neurotransmission of erection cholinergic or not? The histochemical and chemical studies indicate that cholinergic fibres are present in the target organs.

We will first consider the information presented on the effects of acetyl choline. Acetylcholine has been reported to produce a decrease in vascular resistance of the dog penis by Henderson and Roepke (1933) But Dorr and Brody (1967) found no such effect. The discrepancy could be due to differences in e g perfusion pressure as indicated by the studies of Penttilä (1966) and Penttilä and Klinge (1966) In this context it should be mentioned that Dorr and Brody found no definite effect when studying other well known vasodilators and vasoconstrictors. Further the difficulties in evaluating the exact point of action of a drug when perfusing a vascular bed as that of the penis should not be underestimated. In this context it should be recalled that acetylcholine has been reported to relax the cavernous bodies of the rabbit (Hukovic and Bubic 1967). It seems that the hitherto presented data are inconclusive and emphasize the necessity to examine the effect of acetylcholine on isolated inflow resistance vessels and to compare the effect of the drug with that of stimulation of the inhibitory nerves.

The effect of acetylcholine on the retractor penis muscle may appear still more contradictory *In vivo* the transmitter relaxes the muscle (Dale 1914, Oppenheimer 1938) Dale ascribed this effect to the fall in blood pressure caused by acetylcholine This possibility was not considered in Oppenheimer's study in which the blood pressure was not recorded. Therefore Oppenheimer's conclusions of the site of action of acetylcholine are uncertain. Nearly all *in vitro* studies indicate that acetylcholine and other muscarinic agents, if they have any effect, contract the muscle and this effect is blocked by atropine. According to the classic concepts this constitutes the strongest argument against a cholinergic neurotransmission of relaxation of the muscle. However high concentrations of acetylcholine have been reported to relax the muscle (Orlov 1963 b and Gushchin 1965) The mechanism responsible for this effect has not been analyzed. A further discrepancy exists in the literature concerning the effect of nicotine Most investigators have found a stimulant action of this drug but it has also been found to relax the muscle (De Zilwa 1901). Thus there is a need of reinvestigation of the possible relaxation induced by high concentrations of acetylcholine and by nicotine as well as of the target structures of these drugs before acetylcholine can be discarded as the inhibitory

transmitter of the retractor penis muscle. Further the possibility that acetyl choline can inhibit the excitatory adrenergic neurotransmission has to be investigated.

When the pharmacological studies of the neurotransmission of penile erection are considered, two things are striking 1) All authors who have presented evidence in favour of a cholinergic neurotransmission of erection seem to have used preganglionic nerve stimulation. 2) Most authors, both those presenting evidence in favour of a cholinergic neurotransmission as well as those presenting evidence against this idea, have used very high, without doubt often unphysiological frequencies when stimulating the nerves (e.g Bacq 100—300 imp/sec, Luduena and Grigas 20 or 60 imp/sec, Dorr and Brody 20 imp/sec, Hukovic and Bubic 60 imp/sec) These two remarks are important. We now know that autonomic ganglia have muscarinic receptors which are stimulated by an excess of acetylcholine and that atropine has ganglionic blocking properties (e.g Bainbridge and Brown 1960 for further references see Volle and Koelle 1970)

A high frequency of preganglionic nerve stimulation combined as in the experiments of Bacq with physostigmine, is likely to produce stimulation of muscarinic receptors in the ganglia. Hence the suppression by atropine of the effect of pelvic nerve stimulation observed by some authors could entirely be due to a ganglionic effect of the drug. Naturally also the effect of physostigmine reported by many investigators could entirely be of ganglionic origin. However if the studies providing evidence against a cholinergic neurotransmission are taken into consideration, the errors in using a high stimulation frequency are as obvious. Provided the junctional receptors in the target organs are relatively inaccessible to a competitive receptor blocking agent such as atropine (Gaddum and Dale 1930 see also Ursillo and Clark 1956) it might be extremely difficult to record any effect of the blocker if high frequency stimulation yielding an excess of the transmitter in the junctional gaps is applied. Further when the stimulation produces an excess of the transmitter all the receptors may be occupied. Then it might be difficult to record any enhancement produced by a drug preventing the inactivation of the transmitter as physostigmine. This objection could be raised against e g the study of Luduena and Grigas (1966). In view of the results of the present study it is a relevant objection because maximum inhibition of the bull retractor penis is obtained with a very limited number of impulses. In this context it also should be mentioned that Armitage and Burn (1967) considered the hemicholinium experiments of Luduena and Grigas inconclusive. According to Armitage and Burn they had too short a period of exposure to hemicholinium (50—150 min). Thus, neither the pharmacological data supporting a cholinergic neurotransmission of erection nor those against it present definite evidence in either direction. The need of experiments with a compound inhibiting the release of acetylcholine, as botulinum toxin, is obvious. There is also a need to determine the frequency response relationship of inhibitory nerve stimulation and to evaluate the effects of various autonomic drugs when the nerves are stimulated with frequencies in a more physiological range, i.e. not exceeding 15 imp/sec.

Finally attention should be paid to two other compounds tentatively suggested to be the parasympathetic neurotransmitter of erection, namely PGE_1 (Luduena and Grigas 1972) and ATP (Bell 1972 in referring to Burnstock

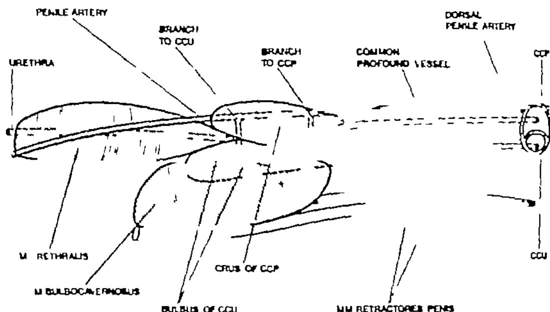


Fig. 2. Schematic drawing of the root of the penis after removal of the ischiocavernosus muscle ccp = corpus cavernosum penis, ccu = corpus cavernosum urethrae.

artery the vessel reaches the root of the penis on the ventral surface of the pubic bone. In the well protected fat filled space between the pubic bone and the thick tunica albuginea of the crus of the *corpus cavernosum penis (ccp)* it gives off the branches serving the cavernous bodies (Figs. 2 and 3). The first branch supplies the *corpus cavernosum urethrae (ccu)* and corresponds to the arteria bulbi urethrae in man. The second branch, which serves the ccp appears to be analogous to the arteria profunda penis in man.

In the bull both branches supplying the cavernous bodies are 1—3 cm long They are paler and more elastic than the stem of the penile artery They usually do not ramify before they have entered their respective cavernous bodies. After entrance into the cavernous bodies the branches rapidly lose their thick muscular walls. In the ccu they open directly into the very large sinusoids typical of this body (Grabowski 1937) In the ccp the two branches soon join each other to form a common profound vessel (Fig 3). The structure of this vessel is quite specific and lacks all the characteristics of an artery The wall of the vessel is thin and full of small openings resembling a strainer Thus the arterial supply of the cavernous bodies of the bovine penis considerably differs from that of man and dog, allowing in a simple manner a rapid filling of the erectile tissue In this connection it should be mentioned that the *corpora cavernosa penis* of the bull appear to be devoid of autonomic nerves and smooth muscle (Klinge and Penttilä 1969)

After giving off the two branches to the cavernous bodies the penile artery continues as the dorsal penile artery towards the distal parts of the penis parallel with and ventral to the dorsal nerve of the penis.

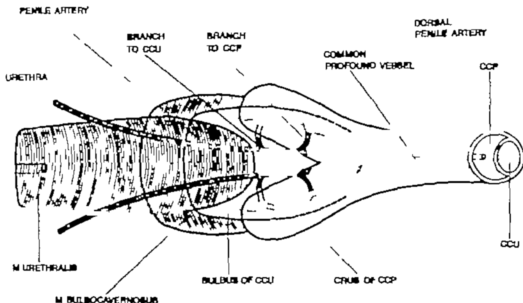


Fig. 3. Schematic drawing of the root of the penis and its arterial supply. Abbreviations as in Fig. 2.

(Star Ltd) theophylline ethylenediamine (Medica Ltd) quinidine sulphate (ACO AB), melatonin (Sigma Chemical Company) creatinine hydrochloride (Sigma Chemical Company) strychnine nitrate (Sigma Chemical Company) picrotoxin (Sigma Chemical Company), and yohimbine hydrochloride (Sandoz AG).

Unless otherwise stated all effects of the drugs were verified on at least three preparations. The results of the present report are based on 219 retractor penis strips and 92 penile artery strips obtained from 156 different bulls.

CHAPTER IV

RESULTS

The scope of the present study is outlined in the previous sections. In addition to known and tentative neurotransmitters, as *e.g.* acetylcholine, noradrenaline, adrenaline, *dopamine*, *5-hydroxytryptamine*, *histamine*, GABA, glycine, aspartic acid, glutamic acid, Substance P, prostaglandins and ATP also other amino acids and nucleotides have been included. Likewise, in addition to the effects of atropine and other autonomic drugs the effects of certain compounds known to interfere with nervous mechanisms, as *e.g.* morphine, picrotoxin, quinidine and strychnine, have been studied. Since atropine-resistant parasympathetic effects have been attributed to the release of plasmakinins (Hilton and Lewis 1956, Fülgraff and Schmidt 1963) the effects of bradykinin, kallikrein and aprotinin were studied. Because posterior pituitary hormone preparations have been reported to relax the bull and dog retractor penis (Klinge 1970c, Luduena and Grigas 1972) and to induce penile engorgement in the dog (Holmquist and Olin 1969) the effects of pure synthetic oxytocin and vasopressin were studied.

The Retractor Penis Muscle

General properties and responses to nerve stimulation

When mounted in the organ bath most preparations exhibited a very low tone during the first 1—3 hours, whereafter the resting tone usually increased and reached a steady level about 2—5 hours after the mounting (Fig. 4). About one fifth of the preparations showed regular spontaneous activity (Figs. 5 and 6). The spontaneous activity could be abolished by increasing the stretch. Spontaneous activity as well as the increase in resting tone seemed to be triggered by field stimulation.

The type of the mechanical response elicited by transmural nerve stimulation was dependent on the stage of contraction, i.e. the resting tone of the muscle strip, and the frequency of the stimuli. During the relaxed stage a contraction was the dominating and characteristic response. When the tone increased the excitatory response proportionally diminished. Simultaneously an inhibitory response immediately following the excitatory one appeared and grew along with the increase in resting tone. The inhibitory response was usually followed by a rather long lasting rebound contraction (Figs. 4

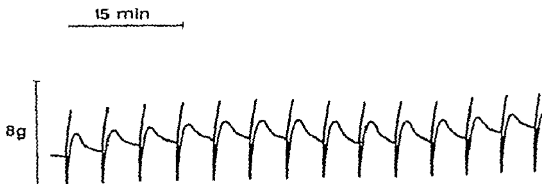


Fig. 4 Retractor penis. Isometric recording. The spontaneous increase in tone is accomplished and a steady state level is reached 4.5 hours after mounting. Due to the medium stage contraction and to the frequency used the responses to nerve stimulation (2 Hz for 10 sec at 5 min intervals) are typically biphasic. Note also the rebound contraction. Duration of the square wave pulses in this and all the following records is 1 msec.

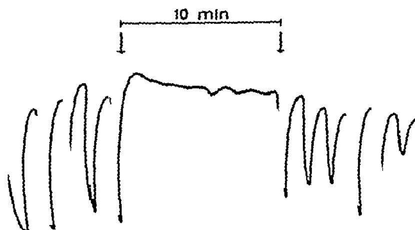


Fig. 5. Retractor penis. Isotonic recording. Load 2.5 g. Magnification 1 4. Spontaneous movements. Continuous field stimulation applied between the arrows with single shocks at 10 sec intervals. The pulse train keeps the strip contracted.

7 19 21 30 and 32 a). The size of this »rebound contraction» was essentially dependent on the depth of the inhibitory response and the resting tone of the muscle. When the strips were in very high tone there was usually no sign of a »rebound contraction». On the other hand, in strips being in low tone the »rebound contraction» could be very pronounced despite a moderate inhibitory response to nerve stimulation. In these cases the combined response to field stimulation almost had the outlook of a biphasic excitatory response (e.g. Fig. 30). In preparations being in very low tone and where the excitatory response had been blocked by e.g. phentolamine the »rebound contraction» could be almost the sole visible response induced by field stimulation. As

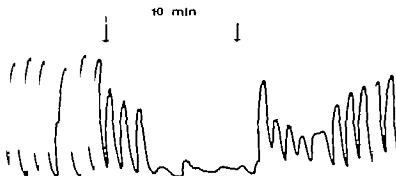


Fig. 6. Retracto penis. Isotonic recording. Same preparation as in Fig. 5 after pretreatment with phentolamine (0.7 μg/ml) which by itself did not interfere with the spontaneous activity. Continuous field stimulation applied between the arrows with single shocks t 10 sec intervals. The response is reversed and the spontaneous activity is suppressed.

judged *from Fig. 7 and from experiments where the excitatory response was* suppressed by *e.g.* phentolamine or enhanced by cocaine (Fig. 19) the rebound contraction was to a certain extent also dependent on the size of the excitatory response.

In sensitive preparations excitatory responses could be evoked by single stimuli (Figs. 8 22 a and b). Further as seen in the rhythmically contracting

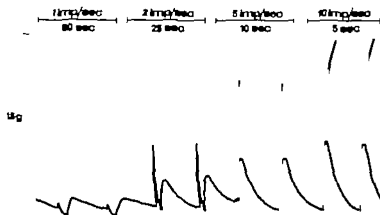


Fig. 7. Retractor penis. Isometric recording. Stimulation with constant number of impulses, i.e. 50, at varying frequencies at 5 min intervals. Note the frequency dependence f the excitatory response.

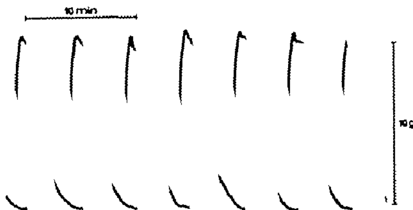


Fig. 8. Retractor penis. Isometric. Responses to single shocks at 5 min intervals. Note the force and duration of the contractions.

preparation in Fig 5, a long train of impulses deliverod at a very low frequency (0 1 imp/sec) could maintain a continuous contraction of the strip. The excitatory response showed a strong dependence on the frequency of stimuli. This is illustrated in Fig. 7 which shows the responses to a constant number of impulses, i.e to 50 delivered at four different frequencies. A frequency response curve of the excitatory response to our usual 10 sec stimulation is presented in Fig. 9 a which shows mean and range of three representative strips from different bulls. As seen in the figure the frequency response curve is fairly flat. However when evaluating this curve it must be kept in mind that the excitatory responses were cut down by the succeeding inhibitory responses which we never were able to selectively abolish or even depress. A true frequency-response curve of the excitation is therefore likely to be considerably steeper That this might be the case is illustrated in Fig. 9 b where the frequency-contraction relationship of a strip taken from the most distal part of the muscle is plotted. This very strip turned out to exhibit almost no inhibitory response to field stimulation. It was, however a rather rigid strip, presumably because of the greater amounts of collagenous tissue in the parts of the muscle close to the insertion. Tissue rigidity as well as paucity of inhibitory nerves might therefore have contributed to the shape of the curve.

The inhibitory response characteristically differed from the excitatory response. This could easily be demonstrated when the preparations were kept in high tone and the excitatory responses were suppressed by an adrenergic neuron blocking agent, as guanethidine, or by an α-receptor blocker as phenoxybenzamine As illustrated in Fig. 10 the inhibitory response to field stimulation is almost the same at 4 8 and 16 imp/sec. In Fig 11 the typical frequency response curve to our usual 10 sec stimulation is presented. The figure gives mean and range of three guanethidine-treated preparations. As seen in the figure the frequency response curve is very steep and maximal inhibition is obtained within 8 imp/sec. This was an almost constant finding Only rarely was a stimulation frequency higher than 8 imp/sec required to

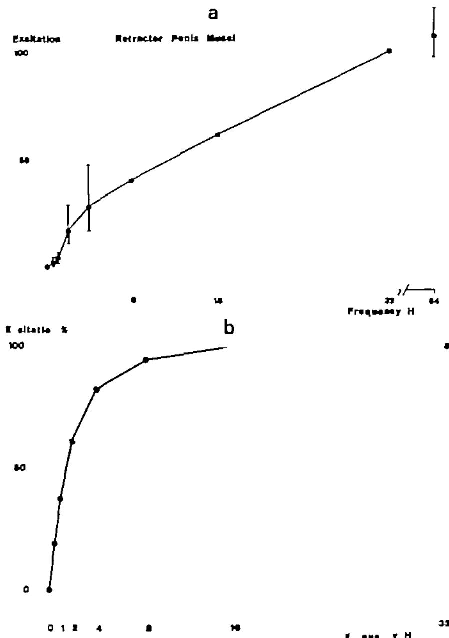


Fig. 9 a. Frequency response curv of isometric contractions to field stimulation. Supr maximal voltage, pulse duration 1 msec, train duration 10 sec. The figure gives mean and range of three trips cut from the middl part of the retractor penis muscle from different bulls. The xperiments were performed 1—2 h fter mounting. The strips were in very low resting tone (0.5—1 g). Here as in the following frequency-response curves the different trains f stimulation were given randomly. Every value from each preparation is the mean of duplicates. The responses are expressed in per cent of the peak response to 32 Hz. Note the fairly flat frequency-response curve. For further comments see the text.
b. Excitatory frequency response curve of preparation cut close to the peripheral insertion of the retractor penis. This preparation proved later (after bretylium) t have almost no inhibitory response to field stimulation. Note the steep frequency-response curve. For further comments see the t t.

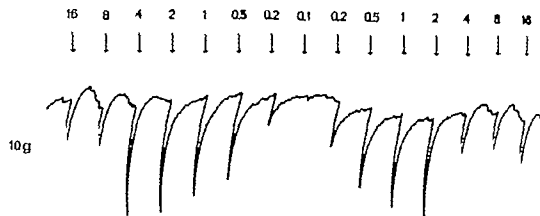


Fig. 10 Retractor penis. Isometric. Pretreatment with bretylium (6 μg/ml). Resting tone about 16 g. Stim. for 10 sec at 5 min interv with varying frequencies. Frequency in Hz indicated by numbers above arrows. Note that if the variance in resting tone is taken into consideration the responses to 8 and 16 Hz are identical and the responses to 4 Hz are very close to the responses to 8 and 16 Hz. *Cf* Fig. 15.



Fig. 11 Frequency response curve of isometric relaxation induced by field stimulation. Supramaximal voltage, pulse duration 1 msec, train duration 10 sec. The figure shows mean and range of three strips cut from the middle part of the retractor penis from different bulls. The strips were pretreated with guanethidine (1.4 μg/ml) in order to abolish the excitatory response and to maintain the strips in high tone (10—16 g). The responses in this and the two following figures are expressed in per cent of the maximum inhibition obtained with nerve stimulation (the response to 8 Hz for 60 sec). Note the very steep frequency response curve. The slightly reduced response to 32 Hz is probably due to a moderate »break through» of the excitatory response seen with this very high frequency. For further comments see the text.

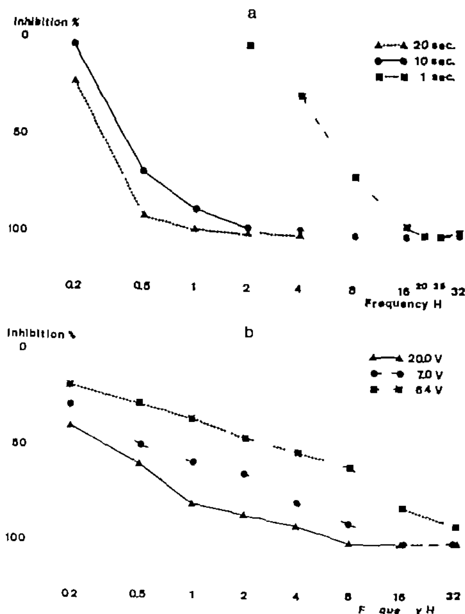


Fig. 12 a. Frequency response curves f inhibitory response to field stimulation of guanethidine-treated (1.4 μg/ml) retractor penis strip. The figure illustrates the effect of changes in duration of the impulse train. Stimulus voltage constant and supramaximal, pulse duration 1 msec. Frequency plotted on a logarithmic scale. An increase in duration of the train to 20 sec, instead of the usual 10 sec, shifts the curve to the left, while decrease of train duration to 1 sec turns the frequency response curve to the right. Note that the same number of impulses, e.g. 2, 4 or 20, causes almost identical degrees of inhibition in the three curves.

b. Frequency-response curves of inhibitory responses to field stimulation of bretylium-treated (9 μg/ml) retractor penis strip. The figure shows the effect of reductions in stimulation voltage, i.e. reduction in number of stimulated nerves. Pulse duration 1 msec, duration of train of stimuli 10 sec. Frequency plotted on logarithmic scale as in a. Reduction in voltage shifts the frequency response curve to the right. With the lowest voltage (6.4 V) maximum inhibition is not reached with the parameters used in the pre-

obtain maximum inhibition. Further if the 8 imp/sec stimulation was extended to periods longer than 10 sec, this usually did not increase the relaxation. In some cases the relaxation rather decreased and stabilized at a slightly higher tone when stimulation was continued (*cf* Fig 38 b).

If the duration of the impulse train was increased from the usual 10 sec, the frequency response curve was shifted towards the left, i.e towards lower frequencies, while a shortening of the duration shifted the curve to the right, i.e towards higher frequencies. This is illustrated in Fig. 12 a where the inhibitory responses to field stimulation at different frequencies and varying durations of the impulse train in a single preparation are plotted on a semilogarithmic scale. A decrease in stimulation voltage, i.e. a decrease in number of stimulated nerve fibres, also shifted the frequency response curve towards the right (Fig 12 b).

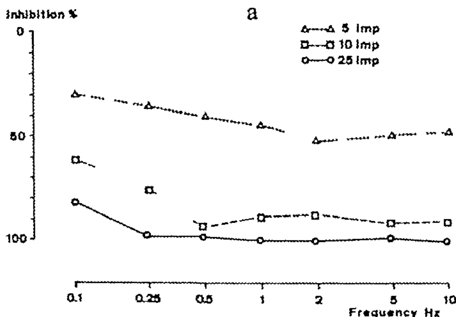
The curve presented in Fig. 12 a also illustrates that with supramaximal stimulation voltage the same total number of impulses produces about the same degree of inhibition. Thus the degree of inhibition to 20 pulses is almost the same when produced by 1 imp/sec for 20 sec, 2 imp/sec for 10 sec or 20 imp/sec for 1 sec. This dependence on the number of the pulses is further illustrated in Figs. 13 a and b. In Fig. 13 a the frequency response curve to 5 10 and 25 impulses delivered at varying frequencies are plotted. The responses are fairly constant in the frequency range of 0.5 to 10 imp/sec. In Fig. 13 b are plotted the impulse response curves from the same preparation at two different frequencies (0.5 and 2 imp/sec). The sigmoid shape of the curves is noticeable It is also seen that in this preparation the almost maximal inhibition was obtained with about 20 impulses. *Thus the inhibitory response to nerve stimulation was characterized by a high dependence on the number of impulses delivered and maximal inhibition was obtained within a limited number of impulses At frequencies of 0.5 to 10 imp/sec and supramaximal stimulation voltage maximum inhibition was as a rule obtained with 80 impulses or less.*

In this connection it should be emphasized that the maximal inhibition obtained by nerve stimulation was not identical with the maximal relaxation of the preparation. The preparations were usually more relaxed when freshly mounted in the organ bath, and when kept in a high tone for study of the inhibitory responses to nerve stimulation further relaxation could always be brought about by e g papaverine or theophyllamine. The inhibitory response to nerve stimulation also involved suppression of the rhythmical spontaneous activity as illustrated in Fig 6 A comparison between Fig 6 and Fig. 5 also shows that when continuous stimulation was performed on untreated preparations the excitatory response dominated. This was usually the case in

---

sented graph. However it could be reached if the duration of the train was increased. Thus with 8 Hz maximum inhibition was obtained in about 90 sec when stimulation voltage was 6.4 V

A difference in shape of the frequency response curves to supramaximal voltage and 10 sec duration of train of stimuli can be seen when a and b are compared (middle curve in a, lowest curve in b). In a the response to 0.2 Hz, i.e. to 2 pulses, is relatively smaller than in b On the other hand, the curve in b is more flat in the higher frequency range, i.e. between 1—8 Hz (10—80 pulses). This difference is probably to a great extent due to the higher resting tone of the strip in b (about 21 g) compared with that of the strip in a (about 11 g) It was a constant finding in this study that responses to very few pulses were much easier to demonstrate in preparations being in very high tone, than in preparations being in low tone.



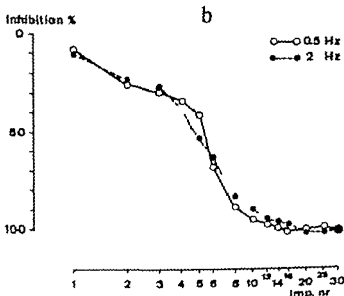


Fig. 13. Inhibitory responses of a retractor penis strip to a fixed number of pulses. The preparation had been treated with phenoxybenzamine (10 μg/ml) for 1 h and thereafter washed repeatedly. A constant high tone (33—23 g) was secured by addition of rela- tively low concentration of barium chloride (5 μg/ml).
a. The responses to 5, 10 and 25 pulses delivered at frequencies between 0.1 and 10 Hz are illustrated. Frequency plotted on logarithmic scale. Note that between 0.5 and 10 Hz the response to certain number of shocks is almost constant regardless of the interval between the individual pulses.
b. Two impulse-response curves of the same preparation ar demonstrated. The number of pulses is plotted on logarithmic scale. The shocks were delivered t two different rates, i.e. 0.5 and 2 Hz. Note the sigmoid shape of the two curves which lmost coincide.

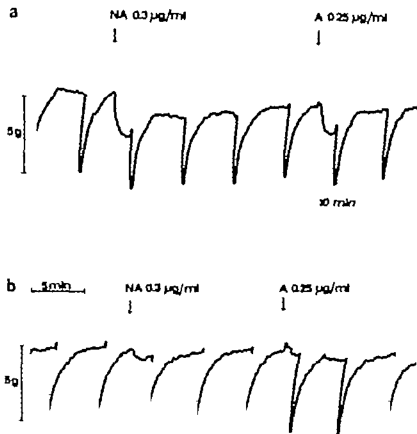


Fig. 16. Retractor penis. Isometric. Pretreated with barium chloride (50 μg/ml) and phentolamine (1.5 μg/ml). Resting tone about 8 g. Stimulation with 0.5 Hz for 10 sec at 5 min intervals. In *a* the preparation is relaxed by noradrenaline (NA) and adrenaline (A). In *b* noradrenaline and adrenaline are almost without effect after addition of propranolol (0.5 μg/ml).

was not stimulated. After this the blockade was irreversible but contractions could still be elicited with frequencies exceeding 20—30 imp/sec. Exposure to 100 ng/ml of phenoxybenzamine for one hour resulted in a decrease by 60—80 per cent of the initial excitatory response. With lower concentrations (50 ng/ml) the usual effect was an immediate suppression of the excitatory response to nerve stimulation, which started to grow again if intermittent stimulation was continued. High concentrations of phenoxybenzamine (1—10 μg/ml) and phentolamine (4—10 μg/ml) usually increased the tone, particularly in field stimulated preparations. Neither of the two α-receptor blocking agents decreased the inhibitory response to nerve stimulation, but due to their effect on the excitatory response and the tone they indirectly increased it.

Propranolol in concentrations up to 2 μg/ml had no distinct effect on the tone of the strip. The responses to nerve stimulation were slightly decreased at very low frequencies (0.1—0.2 imp/sec) but not at higher frequencies (Fig

16b). Two other β-receptor blocking agents with less pronounced local anaesthetic activity practolol (2 μg/ml) and sotalol (2 μg/ml) were without effect on the responses to nerve stimulation and the tone.

Adrenergic neuron blocking agents and other compounds influencing adrenergic mechanisms

Bretylium (3—12 μg/ml) and *guanethidine* (0.7—4.0 μg/ml) effectively blocked the excitatory response to nerve stimulation at frequencies up to 15 imp/sec. Both agents also produced a considerable increase in the tone of the strips and thereby unmasked the inhibitory response (cf Figs. 17 31, 35, 36 and 37). This increase in tone was accelerated by cocaine (100 ng/ml) and

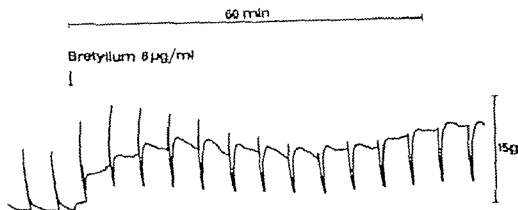


Fig. 17 Retractor penis. Isometric. Stimulation with 3 Hz for 10 sec at 3 min intervals. Bretylium contracts the muscle and initially enhances but subsequently abolishes the excitatory response. The inhibitory response is unmasked. Cf Figs. 27 and 45

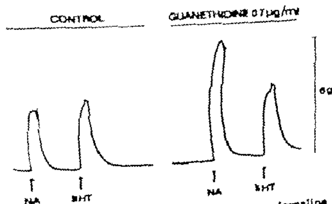


Fig. 18. Retractor penis. Isometric. Contractions to noradrenaline (NA, 100 ng/ml for 30 sec) and 5-hydroxytryptamine (5HT 100 ng/ml for 30 sec). In the presence of guanethidine (right panel) the effect of noradrenaline is enhanced. This preparation is exceptionally sensitive to 5HT

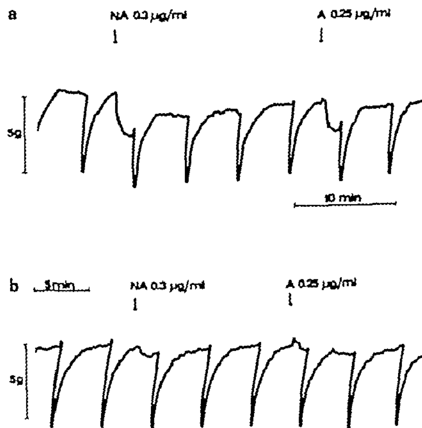


Fig. 16 Retractor penis. Isometric. Pretreated with barium chloride (50 µg/ml) and phentolamine (1.5 µg/ml). Resting tone about 8 g. Stimulation with 0.5 Hz for 10 sec at 5 min intervals. In a the preparation is relaxed by noradrenaline (NA) and adrenaline (A). In b noradrenaline and adrenaline are almost without effect after addition of propranolol (0.5 µg/ml)

was not stimulated. After this the blockade was irreversible but contractions could still be elicited with frequencies exceeding 20—30 imp/sec. Exposure to 100 ng/ml of phenoxybenzamine for one hour resulted in a decrease by 60—80 per cent of the initial excitatory response. With lower concentrations (50 ng/ml) the usual effect was an immediate suppression of the excitatory response to nerve stimulation, which started to grow again if intermittent stimulation was continued. High concentrations of phenoxybenzamine (1—10 µg/ml) and phentolamine (4—10 µg/ml) usually increased the tone, particularly in field stimulated preparations. Neither of the two α-receptor blocking agents decreased the inhibitory response to nerve stimulation, but due to their effect on the excitatory response and the tone they indirectly increased it.

Propranolol in concentrations up to 2 µg/ml had no distinct effect on the tone of the strip. The responses to nerve stimulation were slightly decreased at very low frequencies (0.1—0.2 imp/sec) but not at higher frequencies (Fig.

16b). Two other β-receptor blocking agents with less pronounced local anaesthetic activity *practolol* (2 μg/ml) and *sotalol* (2 μg/ml), were without effect on the responses to nerve stimulation and the tone.

Adrenergic neuron blocking agents and other compounds influencing adrenergic mechanisms

Bretylium (3—12 μg/ml) and *guanethidine* (0.7—4.0 μg/ml) effectively blocked the excitatory response to nerve stimulation at frequencies up to 15 imp/sec. Both agents also produced a considerable increase in the tone of the strips and thereby unmasked the inhibitory response (*cf* Figs. 17, 31, 35, 36 and 37). This increase in tone was accelerated by cocaine (100 ng/ml) and

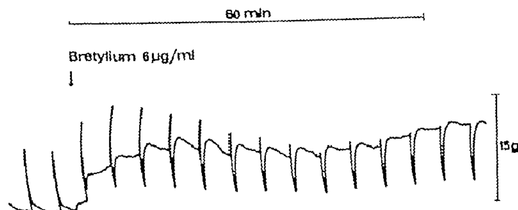


Fig. 17. Retractor penis. Isometric. Stimulation with 3 Hz for 10 sec at 5 min intervals. Bretylium contracts the muscle and initially enhances but subsequently abolishes the excitatory response. The inhibitory response is unmasked. *Cf* Figs. 27 and 45.

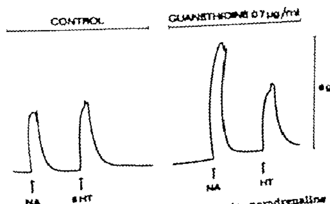


Fig. 18. Retractor penis. Isometric. Contractions to noradrenaline (NA, 100 ng/ml for 90 sec) and 5-hydroxytryptamine (5HT 100 ng/ml for 90 sec). In the presence of guanethidine (right panel) the effect of noradrenaline is enhanced. This preparation is exceptionally sensitive to 5HT





Fig. 19 Retractor penis. Isometric. Stimulation with 1 Hz for 10 sec at 5 min intervals. A low concentration of cocaine gradually increases the excitatory response, the rebound contraction and the tone.

could be counteracted by phentolamine (1 4 μg/ml). It disappeared promptly after washing, whereas the suppression of the excitatory response was highly resistant to washing. The adrenergic neuron blocking agents enhanced the response to exogenous noradrenaline but not to e.g exogenous 5-hydroxytryptamine (Fig 18). Because the adrenergic neuron blocking agents suppressed the excitatory response to nerve stimulation and raised the tone of the strips, studies of the inhibitory response and the effects of relaxing compounds were often performed after pretreatment with guanethidine (1 4 μg/ml)

Cocaine (5—100 ng/ml) enhanced the excitatory response to nerve stimulation as well as the rebound contraction and raised the tone of the preparations (Fig 19) The onset of the effects was accelerated and their magnitude was augmented by increasing concentrations. Similar effects as those of cocaine were obtained with 10 ng/ml of *desipramine* whereas 100 ng/ml initially enhanced the excitatory response to field stimulation but later depressed and finally abolished it. However the rebound contraction grew continuously But also the rebound contraction was suppressed by a still higher concentration (1 μg/ml) Cocaine always enhanced the response to exogenous noradrenaline, whereas it was distinctly reduced by 1 μg/ml of desipramine. Neither of the two inhibitors of neuronal noradrenaline uptake seemed directly to affect the inhibitory response to nerve stimulation.

a Methyldopa in concentrations ranging from 0 2 to 20 μg/ml did not affect the tone of the muscle or its responses to nerve stimulation. When the concentration was raised to 100 μg/ml the excitatory response to field stimulation was slightly reduced after one hour when the usual intermittent stimulation with 10 imp/sec was applied. *Nialamide* (6 μg/ml) had no effect on the tone or on the responses to nerve stimulation during an exposure of 30 min. The other monoamine oxidase inhibitor *β-phenyl isopropylhydrazine* (5 μg/ml) immediately strongly raised the tone of the muscle strip. Presumably this was a direct smooth muscle effect. *Pyrogallol* (5 μg/ml) slightly relaxed the preparation without any obvious effect on the responses to nerve stimulation.

Parasympathomimetic and antimuscarinic agents

Acetylcholine in the concentration range of 1 to 100 ng/ml had no distinct effects on the muscle strips. Higher concentrations (1—100 μg/ml), however usually had clear actions on the preparations. Three different effects could be distinguished 1) A contraction of the strip which usually lasted 10—15 min, whereafter a moderate relaxation occurred (Figs. 20 and 21). These effects were reduced by atropine (1—10 μg/ml) 2) An immediate, sometimes complete supression of the excitatory response to nerve stimulation (Figs. 20 21 and 22a) This effect lasted at least 20 min and was most prominent when the preparations were stimulated with frequencies lower than 3 imp/sec. Atropine (1—10 μg/ml) was able to abolish this effect of acetylcholine (Figs. 22a and b). 3) In concentrations of 10—100 μg/ml acetylcholine produced in some preparations an effect closely resembling that of nerve stimulation, i.e a brisk contraction immediately followed by a profound relaxation. This effect of acetylcholine also resembled that produced by 10 μg/ml of nicotine (see below) It was prevented by hexamethonium (100 μg/ml) but not by atropine.

If the preparations were pretreated with physostigmine the effects of acetylcholine could be obtained with lower concentrations but were qualitatively the same. In preparations contracted by guanethidine 1 ng — 100 μg/ml of acetylcholine had no effect on the inhibitory response induced by nerve stimulation, but in untreated preparations it was indirectly increased due to the transient inhibition of the excitatory response (Fig 20). Acetylcholine had no overt effect on the responses to exogenous noradrenaline (Fig 23) The effects of acetylcholine were the same with the chloride and bromide salts. Corresponding concentrations of sodium bromide were without effect.

Pilocarpine was without effect in concentrations ranging from 1 to 100 ng/ml. Higher concentrations (1—100 μg/ml) slowly raised the tone of the muscle strip. In these concentrations pilocarpine could, like acetylcholine, suppress or abolish the excitatory response to nerve stimulation. This effect of pilocarpine was considerably more long lasting than that of acetylcholine and was also counteracted by atropine (1—3 μg/ml).

Atropine (1—10 μg/ml) had no detectable direct effect on the inhibitory response to nerve stimulation but usually relaxed the preparations and then produced a moderate decrease of the excitatory response. Higher concentrations considerably relaxed the strips. *Scopolamine* exhibited a weaker relaxing effect than atropine and concentrations up to 50 μg/ml could be added to the bath without causing any conspicuous effect on the tone of the strip. The inhibitory response evoked by the usual stimulation for 10 sec was not influenced by these concentrations of scopolamine. This holds true also for the relaxation produced by continuous stimulation for several minutes.

Ganglionic stimulating and blocking agents

Nicotine in concentrations up to 1 μg/ml had no effect on the muscle, but 10 μg/ml produced a rapid transient relaxation (Fig. 24). This was especially prominent in barium-treated preparations (Fig. 25). The relaxation seemed to be facilitated by preceding field stimulation. The speed and slope of the relaxation closely resembled the inhibitory response evoked by field stimulation. But in the absence of barium there was no rapid recontraction. The relaxation w
times preceded by a small but rapid contraction and in those ca

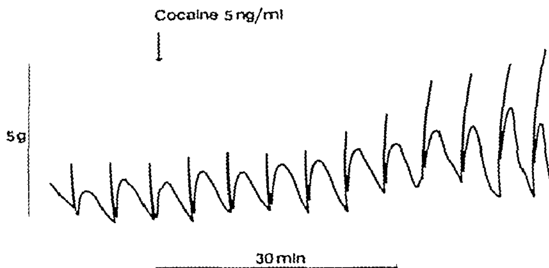


Fig. 19 Retractor penis. Isometric. Stimulation with 1 Hz for 10 sec at 5 min intervals. A low concentration of cocaine gradually increases the excitatory response, the rebound contraction and the tone.

could be counteracted by phentolamine (1.4 μg/ml). It disappeared promptly after washing, whereas the suppression of the excitatory response was highly resistant to washing. The adrenergic neuron blocking agents enhanced the response to exogenous noradrenaline but not to e.g. exogenous 5-hydroxytryptamine (Fig. 18). Because the adrenergic neuron blocking agents suppressed the excitatory response to nerve stimulation and raised the tone of the strips, studies of the inhibitory response and the effects of relaxing compounds were often performed after pretreatment with guanethidine (1.4 μg/ml).

Cocaine (5—100 ng/ml) enhanced the excitatory response to nerve stimulation as well as the rebound contraction and raised the tone of the preparations (Fig. 19). The onset of the effects was accelerated and their magnitude was augmented by increasing concentrations. Similar effects as those of cocaine were obtained with 10 ng/ml of *desipramine* whereas 100 ng/ml initially enhanced the excitatory response to field stimulation but later depressed and finally abolished it. However, the rebound contraction grew continuously. But also the rebound contraction was suppressed by a still higher concentration (1 μg/ml). Cocaine always enhanced the response to exogenous noradrenaline, whereas it was distinctly reduced by 1 μg/ml of desipramine. Neither of the two inhibitors of neuronal noradrenaline uptake seemed directly to affect the inhibitory response to nerve stimulation.

α-Methyldopa in concentrations ranging from 0.2 to 20 μg/ml did not affect the tone of the muscle or its responses to nerve stimulation. When the concentration was raised to 100 μg/ml the excitatory response to field stimulation was slightly reduced after one hour when the usual intermittent stimulation with 10 imp/sec was applied. *Nialamide* (6 μg/ml) had no effect on the tone or on the responses to nerve stimulation during an exposure of 30 min. The other monoamine oxidase inhibitor *β-phenyl-isopropylhydrazine* (5 μg/ml) immediately strongly raised the tone of the muscle strip. Presumably this was a direct smooth muscle effect. *Pyrogallol* (5 μg/ml) slightly relaxed the preparation without any obvious effect on the responses to nerve stimulation.

Parasympathomimetic and antimuscarinic agents

Acetylcholine in the concentration range of 1 to 100 ng/ml had no distinct effects on the muscle strips. Higher concentrations (1—100 μg/ml) however usually had clear actions on the preparations. Three different effects could be distinguished. 1) A contraction of the strip which usually lasted 10—15 min whereafter a moderate relaxation occurred (Figs. 20 and 21). These effects were reduced by atropine (1—10 μg/ml). 2) An immediate sometimes complete supression of the excitatory response to nerve stimulation (Figs. 20, 21 and 22a). This effect lasted at least 20 min and was most prominent when the preparations were stimulated with frequencies lower than 3 imp/sec. Atropine (1—10 μg/ml) was able to abolish this effect of acetylcholine (Figs. 22a and b). 3) In concentrations of 10—100 μg/ml acetylcholine produced in some preparations an effect closely resembling that of nerve stimulation, i.e. a brisk contraction immediately followed by a profound relaxation. This effect of acetylcholine also resembled that produced by 10 μg/ml of nicotine (see below). It was prevented by hexamethonium (100 μg/ml) but not by atropine.

If the preparations were pretreated with physostigmine the effects of acetylcholine could be obtained with lower concentrations but were qualitatively the same. In preparations contracted by guanethidine 1 ng — 100 μg/ml of acetylcholine had no effect on the inhibitory response induced by nerve stimulation, but in untreated preparations it was indirectly increased due to the transient inhibition of the excitatory response (Fig. 20). Acetylcholine had no overt effect on the responses to exogenous noradrenaline (Fig. 23). The effects of acetylcholine were the same with the chloride and bromide salts. Corresponding concentrations of sodium bromide were without effect.

Pilocarpine was without effect in concentrations ranging from 1 to 100 ng/ml. Higher concentrations (1—100 μg/ml) slowly raised the tone of the muscle strip. In these concentrations pilocarpine could like acetylcholine suppress or abolish the excitatory response to nerve stimulation. This effect of pilocarpine was considerably more long lasting than that of acetylcholine and was also counteracted by atropine (1—2 μg/ml).

Atropine (1—10 μg/ml) had no detectable direct effect on the inhibitory response to nerve stimulation but usually relaxed the preparations and [illegible] produced a moderate decrease of the excitatory response. Higher concentrations considerably relaxed the strips. *Scopolamine* exhibited a [illegible] effect than atropine and concentrations up to [illegible]0 μg/ml could be added to the bath without causing any conspicuous effect on the tone of the [illegible] inhibitory response evoked by the usual stimulation for 10 [illegible] enced by these concentrations of scopolamine. This holds [illegible] relaxation produced by continuous stimulation for several [illegible].

Ganglionic stimulating and blocking agents

Nicotine in concentrations up to 1 [illegible] 10 μg/ml produced a rapid transient [illegible] (Fig. 24) [illegible] prominent in barium-treated preparations (Fig. [illegible]) [illegible] facilitated by preceding field stimulation [illegible] closely resembled the inhibitory response [illegible] absence of barium there was [illegible] times preceded by a small but [illegible]

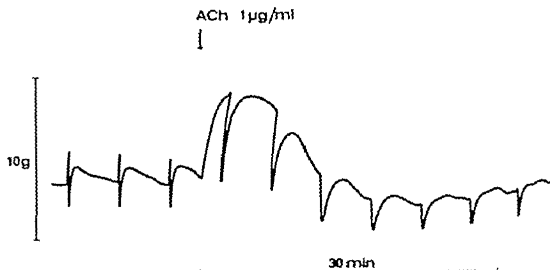


Fig. 20 Retractor penis. Isometric. Stim. 2 Hz for 10 sec at 5 min interv. Pretreated with physostigmine (1 μg/ml). Acetylcholine (ACh) contracts the muscle and abolishes the excitatory response. As a consequence of the suppression of the excitatory response the width of the inhibitory response is increased.

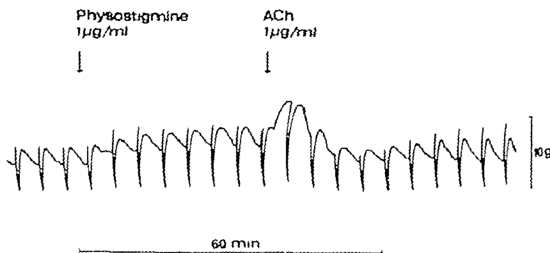


Fig. 21 Retractor penis from castrated bull. Isometric. Stim. 5 Hz for 10 sec at 5 min interv. Physostigmine initially increases the tone and enhances the excitatory response. Acetylcholine (ACh) contracts the muscle and transiently suppresses the excitatory response.

of nicotine mimicked the biphasic response induced by nerve stimulation. The relaxation produced by nicotine was completely prevented by hexamethonium (100 μg/ml) mecamylamine (0.5 μg/ml) TMA (5 μg/ml) McN A 343 (10 μg/ml), d tubocurarine (10 μg/ml) and lidocaine (100 μg/ml). It further was blocked by itself i.e. at least 30 min had to elapse before it could be reproduced. It was only slightly reduced by 1 μg/ml of d tubocurarine but was not influenced by atropine (2 μg/ml) or pilocarpine (100 μg/ml) nor was it affected by a low concentration (0.8 μg/ml) of DMPP. It was totally prevented by propranolol

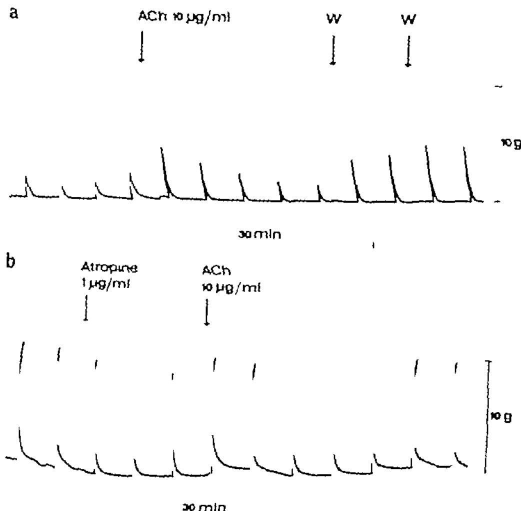


Fig. 22. Retractor penis. Isometric. Stimulated with single shocks at 5 min interv. In a acetylcholine suppresses the excitatory response. In b after repeated washings, atropine prevents the suppression of the excitatory response by acetylcholine. Cf. Figs 46 and 47

(2 µg/ml), partly reduced by practolol (2 µg/ml), but unaffected by [illegible] (2 µg/ml). It was further found that pretreatment with hydrocortisone (60 µg/ml) creatinine (10 µg/ml), melatonin (10 µg/ml) or inosine (10 µg/ml) [illegible] not affect the relaxation.

In the concentration of 100 µg/ml nicotine raised the tone of the muscl[illegible] and slightly reduced the excitatory response induced by field stimulati[illegible] the concentration was increased to 1 mg/ml a profound and long lasting [illegible] tion occurred. Washing performed during this relaxation resulted in [illegible] and powerful contraction which was almost unaffected by pret[illegible] phentolamine (1.5 µg) or phenoxybenzamine (1 µg/ml) and also by [illegible] (2 µg ml). These effects of nicotine were not prevented by 100 [illegible] methonium. The inhibitory responses to field stimulation wer[illegible] directly influenced by the nicotine concentrations studied [illegible]

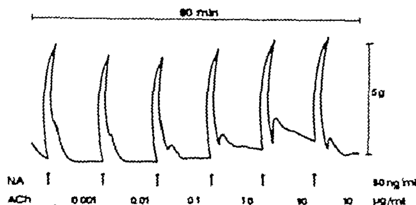


Fig. 23. Retractor penis. Isometric. Contractions produced by the same concentration of noradrenaline (NA, 50 ng/ml for 60 sec) in the presence of increasing concentrations of acetylcholine (ACh). First contraction control. Even in high concentrations acetylcholine is without significant influence on the contractions.

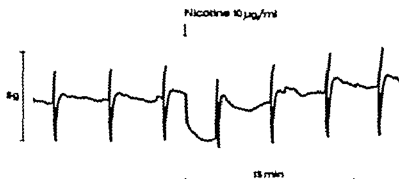


Fig. 24. Retractor penis. Isometric. Resting tone about 6 g. Stim. 10 Hz for 10 sec at 5 min interv. *Nicotine (10 µg/ml) induces a rapid relaxation. Cf Figs. 25, 26 and 48.*

of nicotine this also applies to the contractions produced by exogenous noradrenaline or 5-hydroxytryptamine.

All the above effects seemed to be produced by nicotine itself since no effects were obtained with the corresponding concentrations of sodium bitartrate.

Also with *DMPP* (1.2 µg/ml) a brisk response resembling that evoked by nerve stimulation could be obtained (Fig. 26). However the predominant effects of DMPP were a blockade of the excitatory response to field stimulation, an increase in tone of the preparation and an unmasking of the inhibitory response (Fig 27). These effects always seen with 6 µg/ml closely resembled those produced by guanethidine and bretylium. The rise in tone caused by DMPP was in part prevented by phentolamine (0.75 µg/ml). Like guanethidine DMPP (0.6 µg/ml) potentiated the response to exogenous noradrenaline without influencing the response to e.g. exogenous histamine.

The *TMA* concentration of 0.05 µg/ml was without effect whereas 0.5 µg/ml slightly but distinctly enhanced the excitatory response to field stimulation. This effect was further augmented by 5 µg/ml. The highest concentration used was 50 µg/ml and it moderately raised the tone of the strip and depressed the

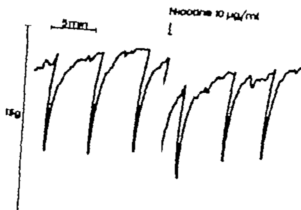


Fig. 25. Retractor penis. Isometric. Pretreatment with barium chloride (50 µg/ml) Resting tone about 28 g. Stim. 0.2 Hz for 10 sec at 5 min interv. Nicotine (10 µg/ml) produces a profound but transient relaxation.

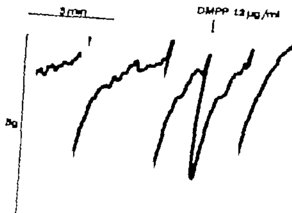


Fig. 26. Retractor penis. Isometric. Pretreatment with barium chloride (50 µg/ml). Resting tone about 16 g. DMPP (1.2 µg/ml) induces a brisk response mimicking those elicited by nerve stimulation (0.8 Hz for 10 sec at 5 min interv.). *Cf* Figs. 24, 25 and 48.



Fig. 27. Retractor penis. Isometric. Stim. 4 Hz for 10 sec at 5 min interv. DMPP (6 µg/ml) increases the tone and abolishes the excitatory response. As a consequence of this the inhibitory response is unmasked. *Cf* Fig. 17

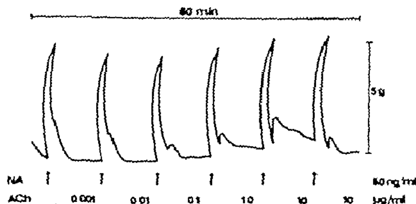


Fig. 23 Retractor penis. Isometric. Contractions produced by the same concentration of noradrenaline (NA, 50 ng/ml for 60 sec) in the presence of increasing concentrations of acetylcholine (ACh) First contraction control. Even in high concentrations acetylcholine is without significant influence on the contractions.

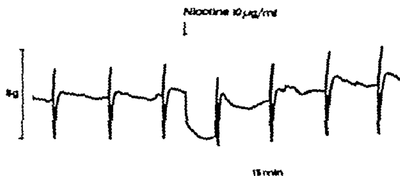


Fig. 24. Retractor penis. Isometric. Resting tone about 6 g. Stim. 10 Hz for 10 sec at 5 min interv Nicotine (10 μg/ml) induces a rapid relaxation. Cf Figs. 25, 26 and 44.

of nicotine this also applies to the contractions produced by exogenous noradrenaline or 5-hydroxytryptamine

All the above effects seemed to be produced by nicotine itself since no effects were obtained with the corresponding concentrations of sodium bitartrate.

Also with *DMPP* (1.2 μg/ml) a brisk response resembling that evoked by nerve stimulation could be obtained (Fig. 26). However the predominant effects of DMPP were a blockade of the excitatory response to field stimulation, an increase in tone of the preparation, and an unmasking of the inhibitory response (Fig 27) These effects always seen with 6 μg/ml closely resembled those produced by guanethidine and bretylium. The rise in tone caused by DMPP was in part prevented by phentolamine (0.75 μg/ml). Like guanethidine, DMPP (0 6 μg/ml) potentiated the response to exogenous noradrenaline without influencing the response to e.g exogenous histamine.

The *TMA* concentration of 0 05 μg/ml was without effect whereas 0.5 μg/ml slightly but distinctly enhanced the excitatory response to field stimulation. This effect was further augmented by 5 μg/ml. The highest concentration used was 50 μg/ml and it moderately raised the tone of the strip and depressed the

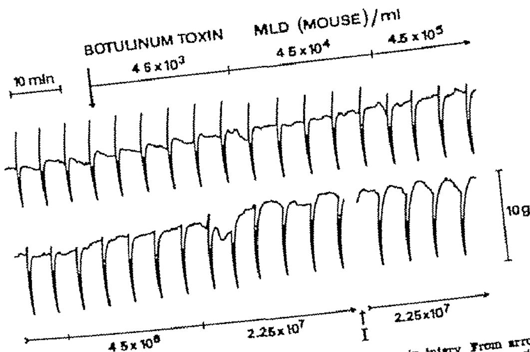


Fig. 28. Retractor penis. Isometric. Stim. 3 Hz for 10 sec at 5 min interv From arrow on increasing concentrations of botulinum toxin. Upper and lower panel are successive records. I = interval of 25 min. Note the decrease of the excitatory response, while the inhibitory response remains unimpaired.

decrease was seen in the inhibitory response. The decrease of the excitatory response seemed, however not to have been principally due to the hemicholinium *per se* but rather to the intense stimulation, because the same stimulation pattern decreased the excitatory response in untreated preparations too

Physostigmine (1—10 µg/ml) raised the tone of the muscle strips. The inhibitory response evoked by field stimulation was not directly influenced, whereas the excitatory response was in most preparations slightly increased tran mently (Fig 21). The contraction elicited by physostigmine was partly prevented by atropine (2 µg/ml) or phentolamine (7.5 µg/ml)

Botulinum toxin in increasing concentrations (4.5 × 10³—2.25 × 10⁷ MLD/ml) did not in the course of more than three hours influence the inhibitory response to nerve stimulation. The higher concentrations of the broth preparation depressed the excitatory response and raised the muscle tone (Fig 28).

Histamine 5-hydroxytryptamine and agents blocking their receptors

Histamine (0.1—1 µg/ml) and *5-hydroxytryptamine* (25 ng—1 µg/ml) contracted the muscle in a dose-dependent manner without directly affecting the inhibitory response to field stimulation. In some preparations the excitatory response was transiently slightly increased by each compound. *Mepyramine* relaxed the muscle already in the concentration 0.07 µg/ml The strips were also slightly relaxed by 1—2 µg/ml of *phenbenzamine* whereas they were contracted by 0.1—1.2 µg/ml of *methysergide* None of these three compounds seemed directly to interfere with the relaxation elicited by transmural nerve

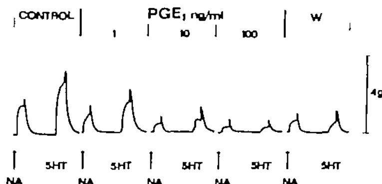


Fig. 29 Retractor penis. Isometric. The contractions produced by noradrenaline (NA, 50 ng/ml for 90 sec) and 5-hydroxytryptamine (5HT 25 ng/ml for 90 sec) are equally reduced by increasing concentrations of PGE$_1$ W = 30 min later after washing. This preparation exhibited exceptionally high sensitivity to 5HT

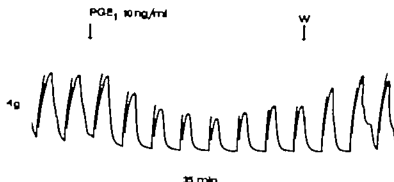


Fig. 30. Retractor penis. Isometric. Stim. 2 Hz for 10 sec at 5 min interv PGE$_1$ attenuates the excitatory response and the rebound contraction. W = washing. *Cf* Fig. 51.

stimulation. *Cyproheptadine* (0.2—20 μg/ml) relaxed the muscle and depressed the excitatory response to field stimulation but did not directly affect the inhibitory response. In a concentration of 0.2 μg/ml cyproheptadine totally blocked the responses to histamine (1 μg/ml) and 5-hydroxytryptamine (1 μg/ml) whereas the response to an equipotent concentration of noradrenaline (50 ng/ml) was reduced by about 50 %.

Prostaglandins prostaglandin antagonists and inhibitors of prostaglandin synthesis

PGE$_1$ (1—100 ng/ml) produced a clear-cut relaxation in contracted muscle strips. It further decreased in a dose-dependent way the responses to exogenous noradrenaline and *e.g* 5-hydroxytryptamine (Fig 9) The maximal inhibition was achieved with 0 1 μg/ml, *i.e* a further increase in concentration (1 μg/ml) did not produce stronger inhibition. The excitatory responses evoked by field stimulation were also suppressed by PGE$_1$ (Fig 30) This could be counteracted in part by doubling the calcium chloride concentration in the organ

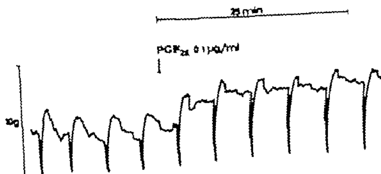


Fig. 31. Retractor penis. Isometric. Pretreated with guanethidine (1.4 µg/ml). Stim. 4 Hz for 10 sec at 5 min interv. PGF$_{2\alpha}$ in contrast to PGE$_1$, increases the tone of the muscle.

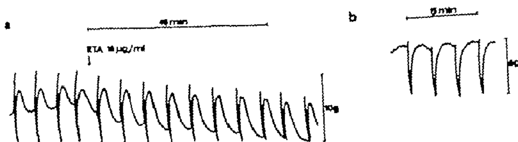


Fig. 32. Retractor penis. Isometric. Stim. 3 Hz for 10 sec at 5 min interv. In a ETA relaxes the muscle strip without overtly influencing the responses to nerve stimulation. In b after 8 hours' exposure to ETA (10 µg/ml) the tone of the strip is raised with guanethidine (1.4 µg/ml). Note the persistence of the inhibitory responses.

bath. The relaxations produced by PGE$_1$ were resistant to propranolol (0.5 µg ml), which fully blocked relaxations of identical size induced by isoprenaline. The prostaglandin antagonist SC 19220 (20 µg/ml) did not counteract relaxations induced by PGE$_1$. PGE$_2$ (1—100 ng/ml) had similar but weaker effects than PGE$_1$. PGF$_{2\alpha}$ (0.1—1 µg ml) raised the tone of the muscle (Fig. 31) and slightl[illegible] enhanced the excitatory response to field stimulation. None of the abo[illegible] prostaglandins appeared to affect directly the inhibitory response to nerve stimulation. SC 19220 (2—[illegible]0 µg/ml) produced a moderate relaxation of the [illegible] and slightly reduced the excitatory response to field stimulation but the inhib[illegible]tory response was unimpaired.

Of the compounds known to inhibit the synthesis of prostaglandins [illegible] (10 µg/ml) relaxed the muscle without directly affecting the excitatory [illegible] elicited by transmural nerve stimulation (Fig. 32 a). Neither did [illegible] influence the inhibitory response (Fig. 32 b). Also *indomethaci[illegible]* [illegible] was without effect on the responses to nerve stimulation, [illegible] stimulated for more [illegible] three hours with 5 or 20 imp/sec for 10 [illegible] intervals exhibited [illegible] identical with those before application [illegible]

Amino acids

The tone of the muscle was slightly decreased and the excitatory response brought about by nerve stimulation was somewhat cut down by 0.1 μg/ml of *GABA* but these effects were not augmented by 1 μg/ml. The concentrations of 10—20 μg/ml, on the other hand, increased the tone of the strips. The inhibitory responses were unaffected. *Glycine* had no effect on the responses to field stimulation but 0 1 μg/ml slightly relaxed the muscle and also here the effect was not rendered greater by 1 μg/ml, while 10—20 μg/ml moderately contracted the preparations. The effects of *glutamic acid* (0 1—20 μg/ml) closely resembled those of GABA. *Aspartic acid (0 1—20 μg/ml) had no effect.* When studied in the concentration of 10 μg/ml the following amino acids were without any distinct effect. *alanine valine leucine isoleucine serine threonine phenylalanine tyrosine N acetyltyrosine tryptophan cysteine methionine proline hydroxyproline glutamine asparagine histidine arginine* and *lysine*

Nucleosides and nucleotides

Adenosine (10 μg/ml) increased the tone and moderately enhanced the excitatory response without influencing the inhibitory response to nerve sti mulation. *Cytidine guanosine* and *inosine* had no effect on the preparation in the concentration of 10 μg/ml.

ATP (1—10 μg/ml) always contracted the muscle strip This effect was partially and reversibly reduced by quinidine (80 μg/ml). It was not abolished by phentolamine (0 75 μg/ml) *ADP* (2—5 μg/ml) had similar effects as ATP although they were somewhat weaker Also *AMP* (2—20 μg/ml) increased the tone of the preparations but its effect was still weaker than that of ADP The effects of the adenosine nucleotides were characterized by considerable tachyphylaxis. No overt effect on the responses to field stimulation was observed. *Cyclic AMP* in concentrations ranging from 0 001 to 10 μg/ml had no distinct effect, whereas 100 μg/ml slightly depressed the excitatory response without influencing the tone or the inhibitory response. *CTP* (0 01—5 μg/ml) *ITP* (0 01—10 μg/ml) *GTP* (0 01—5 μg/ml) *GDP* (5 μg/ml) and *GMP* (5 μg/ml) were without any effect on the muscle strips.

Peptides

The strips were contracted by very low concentrations of *bradykinin* (0.5 ng/ml) *eledoisin* (10 ng/ml) and *Substance P* (50 ng/ml) as illustrated in Figs. 33 34 and 35 These peptides did not affect the inhibitory response to field stimulation but often initially enhanced the excitatory response. Since the solvents of the bradykinin and the eledoisin preparations considerably relaxed the muscle strips it is likely that the retractor penis is contracted by still lower concentrations of these peptides. No definite effect was obtained with pure *angiotensin amide* until concentrations of 0 05—0 1 mg/ml were reached. Then some preparations were slightly relaxed, but the responses to nerve stimulation were unaffected. With the commercial preparation Hypertensine® moderate relaxations were obtained with lower concentrations of angiotensin amide. They were probably due, however to the *mannitol* present in the preparation since mannitol itself relaxed the strips. The mere synthetic

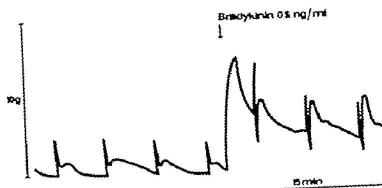


Fig. 33. Retractor penis. Isometric. Stim. 2 Hz for 10 sec at 5 min interv. A low concentration (0.5 ng/ml) of bradykinin contracts the preparation and initially enhances the excitatory response.

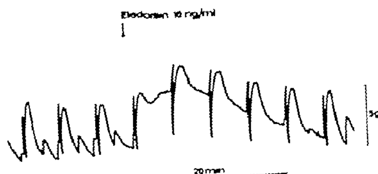


Fig. 34. Retractor penis. Isometric. Stim. 2 Hz for 10 sec at 5 min interv. Also eledoisin produces a considerable rise in the tone f the muscle.

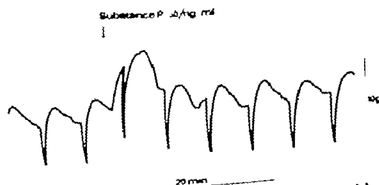


Fig. 35. Retractor penis. Isometric. Pretreatment with guanethidine (1 μg/ml). Stim. 4 Hz for 10 sec at 5 m in erv. Substance P (50 ng/ml) further incre es the t e

Of other ions the effects of *magnesium* and *potassium* were studied. A increase of the original magnesium chloride concentration (100 μg/ml) t 400 μg/ml resulted in a rapid and long lasting relaxation. Doubling of th original potassium chloride concentration (200 μg/ml) produced a rapid an profound but very transient relaxation.

Ergotamine (5 μg/ml) vigorously contracted the muscle strips, whereafte they could not be relaxed by adrenaline (0.5 μg/ml) or isoprenaline (100 μg/ ml) In relaxed preparations the inhibitory response induced by field stimula tion was unmasked. *Papaverine* (0 1—1 μg/ml) profoundly relaxed the muscl and depressed the excitatory response. Also *theophylline* (0 08—8 μg/m strongly relaxed the muscle and attenuated the excitatory response withou directly facilitating the inhibitory response

Morphine in concentrations ranging from 0 008 to 0 8 μg/ml had no effec on the tone of the strips but it was slightly decreased by still higher concentra tions (8—80 μg/ml) None of these concentrations affected the responses evoke by nerve stimulation.

Quinidine in a concentration of 0.8 μg/ml slowly suppressed the excitator response to nerve stimulation. When the concentration was increased t 80 μg/ml the excitatory response was abolished. This effect seemed irreversibl since the excitatory response did not reappear despite repeated washing in preparations exposed to a high concentration of quinidine. However there was only a slight reduction of the muscle tone and the inhibitory response

Picrotoxin and *strychnine* in concentrations up to 10 μg/ml exerted no over influence on either the tone or the responses to field stimulation. *Yohimbine* (1 μg/ml) moderately relaxed the strips and slightly decreased the excitatory response to nerve stimulation. These effects became more pronounced with 10 μg/ml but they were never dramatic. No clear effect was seen on the inhibitory response to field stimulation.

The effects of *melatonin* (10 μg/ml) and *creatinine* (10 μg/ml) were studied in preparations contracted by barium chloride (50 μg/ml) Neither of these compounds influenced the tone of the muscle or the responses elicited by field stimulation. *Chlorobutanol* and *ethanol* which were present in the solvents of the various commercial peptide preparations relaxed the muscle (*cf* Fig 36).

The Penile Artery

General properties and responses to nerve stimulation

When mounted in the organ bath the helical strips of the penile artery usually exhibited a very low tone during the first two hours. Then the tone generally increased rather rapidly and to a relatively greater degree than in the strips of the retractor penis muscle. The spontaneous tone of the arterial strips was rarely as constant as that of the retractor penis preparations. Prominent spontaneous movements were observed in about 10 per cent of the arterial preparations. In common with the retractor penis strips the quality of the response to field stimulation was dependent on the tone of the preparations.

The frequency of the field stimulation had, however less influence on the quality of the responses of the arterial strips. Changes in stimulation frequency mainly altered the magnitude of the responses not their shape. Preparations in low tone showed only excitation and those in a very high tone only inhibition (cf Fig. 54) As in the retractor penis muscle the excitation preceded the inhibition. The speed of both types of response was slower in the arterial preparations than in the retractor penis. This was possibly due to the greater elasticity of the arterial preparations. In general the peak of the excitatory response occurred some seconds after cessation of a 5—10 sec pulse train, whereafter the inhibitory response started to grow Rebound contractions (e.g Figs. 51 and 53) following the inhibitory response to field stimulation were rare in the arterial strips. Usually recovery of tone after inhibition was rather slow (e.g Figs. 44 46 and 50) Only very few arterial preparations responded to single shocks (Fig 48)

A clear difference between the arterial strips and the retractor penis was revealed when a frequency response analysis was performed. With the usual stimulation for 10 sec both types of response increased up to 64 imp/sec which was the highest frequency used (Figs. 39 and 40) Both graphs give the mean and range of three representative strips cut from the branch serving the ccu (cf Figs. 2 and 3). The frequency analysis of the excitatory response was performed one hour after mounting in the bath. The frequency analysis of the inhibitory response was performed about three hours after mounting, when the preparations were spontaneously in high tone. Guanethidine was added before the examination of the inhibitory response. As seen in the figures, the inhibitory frequency response curve is almost the reverse image of the excitatory frequency response curve. When evaluating the curves it must be kept in mind that the cutting as such implies a change in and also a damage to the tissue. But, under the conditions used the penile artery preparation never showed the same distinct and easily obtained maximum inhibition which was so typical of the retractor penis. In fact, with an increasing number of impulses the inhibitory response increased until the preparation became almost flaccid (Fig. 41).

In essence the above description applies to all the arterial strips used in our study i.e. strips cut from the branches serving the cavernous bodies, the segment between these branches and the segment distal to the branch supplying the ccp. But there were some slight differences strips cut from the branches serving the cavernous bodies usually showed quantitatively larger responses than the more rigid strips from the stem of the artery On the other hand, the latter strips generally exhibited a more stable resting tone. They were therefore preferentially chosen for long term experiments, as for instance when the effects of hemicholinium were studied. A clear difference was noted between arterial strips from young and old bulls. Strips taken from calves always developed considerably less force than those taken from old bulls. However this difference seemed to be entirely quantitative. No qualitative difference in responses to field stimulation was noted between penile arteries from bulls weighing about 200 kg and similar strips from bulls weighing about 1000 kg. Nor was there any qualitative difference between arterial strips from bullocks when compared with strips from uncastrated animals. But strips from castrated bulls were as weak as those obtained from calves. The force developed by the arterial strip seemed roughly to be correlated with the thick

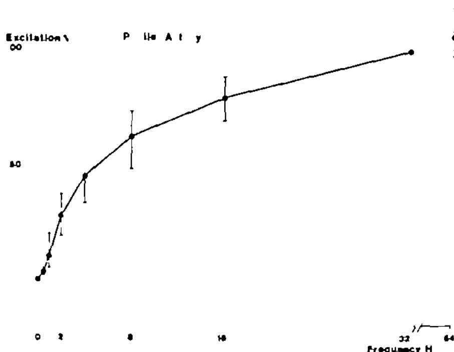


Fig. 39 Frequency response relationship of isometric peak tension developed by helical strips of the penile artery when intramural nerves were stimulated. Supramaximal voltage, pulse duration 1 msec, train duration 10 sec. The figure gives mean and range of three strips cut from the branch to the ccu. All the strips are from different bulls. The responses are expressed in per cent of the response to 32 Hz. Individual values are means of duplicates. The experiments started about 1 h after mounting. The strips were in low tone (0.2—0.4 g)

ness of the arterial wall. Strips which did not spontaneously develop a high tone were treated with barium chloride or ergotamine if the inhibitory responses were to be studied.

In order to make clear that the inhibitory response to transmural nerve stimulation was a specific feature of the penile artery and not a phenomenon shared by all bovine arteries, we examined helical strips from the anterior mesenteric artery the spermatic artery the splenic artery the dorsal metatarsal artery (Fig 42 versus 43) and branches of the internal pudendal artery serving the distal rectum and the region of the anal orifice and the urinary bladder We were not able to obtain any sign of an inhibitory response to field stimulation in any of these arteries in spite of treatment with barium chloride and guanethidine. All of them showed an excitatory response which could be blocked by guanethidine. Further when serial segments of the penile artery were analyzed it was found that, although weak, an inhibitory response to field stimulation could be seen in strips taken 4—6 cm, but not more, proximal to the branch supplying the ccu. Inhibitory responses were always obtained in the segments distal to the branch serving the ccp i.e in the dorsal penile artery Finally it should be mentioned that strips cut longitudinally from the penile artery exhibited very faint responses to nerve stimulation as well as to drugs.

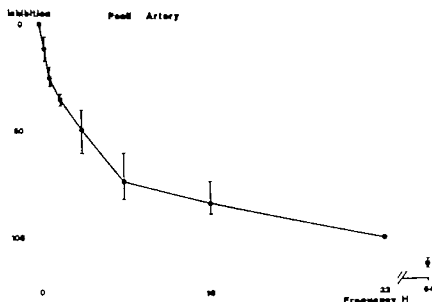


Fig 40. Frequency response relationship of drop in isometric tension of spiral strips of the penile artery when subjected to transmural nerve stimulation. Supramaximal voltage, pulse duration 1 msec, train duration 10 sec. The figure shows mean and range of three strips taken from the branch to th ccu. All the strips are from different speci mens. Two strips are identical in this curve and the curve in Fig. 39 As in Figs. 9, 11 and 39, every value from each preparation is th mean of two determinations and the different trains were pplied in random order t 5 min intervals. The responses are expressed in per cent of the response to 32 Hz. The experiment started 3—5 h after mounting when the strips had spontaneously developed a high tone (3.5—6 g) Guanethi dine (1.4 µg/ml) was added shortly before recording started. Note that the inhibitory frequency-response curve in this figure is almost the reversed imag of the excitatory frequency response curve in Fig. 30 although somewhat steeper This circumstance might be explained by the fact that the competing excitatory response was suppressed by guanethidine in the inhibitory frequency response analysis, whil nothing could be done in order to suppress the competing inhibitory response in the excitatory frequency response analysis. N t also that 64 Hz produce still greater relaxation than 32 Hz.

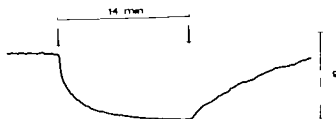


Fig. 41 Penile artery segment just proximal to the branch to the ccu. Isometric. Pre-treatment with barium chloride (100 µg/ml) and guanethidine (1 4 µg/ml). Between rrows continuous timulation with 0.5 Hz. Note that the strip continues to relax during the whol stimulation period. Cf Figs. 38 and b

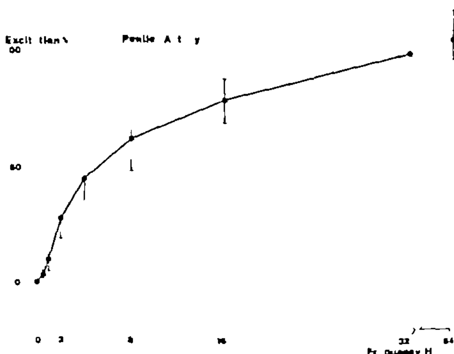


Fig. 39. Frequency response relationship of isometric peak tension developed by helical strips of the penile artery when intramural nerves were stimulated. Supramaximal voltage, pulse duration 1 msec, train duration 10 sec. The figure gives mean and range of three strips cut from the branch to the ccu. All the strips are from different bulls. The responses are expressed in per cent of the response to 32 Hz. Individual values are means of duplicates. The experiments started about 1 h after mounting. The strips were in low tone (0.2—0 4 g).

ness of the arterial wall. Strips which did not spontaneously develop a high tone were treated with barium chloride or ergotamine if the inhibitory responses were to be studied.

In order to make clear that the inhibitory response to transmural nerve sti mulation was a specific feature of the penile artery and not a phenomenon shared by all bovine arteries, we examined helical strips from the anterior mesenteric artery the spermatic artery the splenic artery the dorsal metatarsal artery (Fig 42 versus 43) and branches of the internal pudendal artery serving the distal rectum and the region of the anal orifice and the urinary bladder We were not able to obtain any sign of an inhibitory response to field stimulation in any of these arteries in spite of treatment with barium chloride and guanet hidine. All of them showed an excitatory response which could be blocked by guanethidine. Further when serial segments of the penile artery were analyzed it was found that, although weak, an inhibitory response to field stimulation could be seen in strips taken 4—6 cm, but not more, proximal to the branch supplying the ccu. Inhibitory responses were always obtained in the segments distal to the branch serving the ccp i.e. in the dorsal penile artery Finally it should be mentioned that strips cut longitudinally from the penile artery exhibited very faint responses to nerve stimulation as well as to drugs.



Fig. 44. Penile artery branch to ecu. Isometric. Spontaneously in high tone. Stim. 8 Hz for 10 sec at 5 min interv Tetrodotoxin obliterates the inhibitory response to field stimulation. Possibly as consequence of this the tone is increased. In this very preparation the inhibitory response to field stimulation appeared to be more sensitive to tetrodotoxin than the excitatory response. Note also the prompt reappearance of the inhibitory response after washing. Cf Fig. 14.

Sympathomimetic amines and adrenergic receptor blocking agents

Adrenaline and *noradrenaline* (50 ng — 2 μg/ml) contracted the penile artery in a dose-dependent manner This effect of the two catecholamines was abolished by phenoxybenzamine (0 1—10 μg/ml) and phentolamine (0 1—10 μg/ml). As in the retractor penis, no overt reduction of the inhibitory response to nerve stimulation was seen when the peak of the catecholamine-induced contraction was reached. But before the peak had been reached the inhibitory response to nerve stimulation was usually decreased. A reduction of the inhibitory response to field stimulation was also seen when exceptionally high concentrations (50 μg/ml) of the catecholamines were used. However in these respects adrenaline and noradrenaline did not differ from other contracting agonists as histamine vasopressin, acetylcholine or prostaglandins (cf Figs. 46b and 49)

If the arterial strips were pretreated with an α-receptor blocking agent and kept in tone with barium or ergotamine a relaxation could be produced with adrenaline or noradrenaline (0 1—1 μg/ml) *Isoprenaline* (0 01—1 μg/ml) also relaxed contracted preparations and suppressed rhythmic activity in untreated or barium-treated strips. These effects were prevented by propranolol (1—2 μg/ml)

The excitatory response to field stimulation was inhibited by *phenoxybenzamine* (1—10 μg/ml) and *phentolamine* (1—10 μg/ml) As in the case of the retractor penis, an effective phenoxybenzamine-induced blockade of the excitatory response elicited by field stimulation required a concentration of 10 μg/ml and an exposure of one hour during which the preparation was not stimulated. This pretreatment produced an irreversible blockade unless very high frequencies (20—30 imp/sec) were used. When similar treatment was performed with 100 ng/ml of phenoxybenzamine the excitatory responses decreased to about 20—40 per cent of their original height. Because the α-receptor blocking agents usually increased the tone of the preparations and suppressed the excitatory response they unmasked the inhibitory response.

Propranolol (1—5 μg/ml) and *sotalol* (1—5 μg/ml) did not overtly affect the tone of the muscle strips or the response to transmural nerve stimulation.

Adrenergic neuron blocking agents and other compounds influencing adrenergic mechanisms

Bretylium (3—12 μg/ml) and *guanethidine* (0 7—4 μg/ml) blocked the excitatory response to nerve stimulation (Fig 45) Both agents sometimes increased the tone of the arterial preparations, but this effect was never as pronounced as in the retractor penis strips. Neither of them seemed to directly affect the inhibitory response to field stimulation but they uncovered it as a consequence of the suppression of the excitatory response.

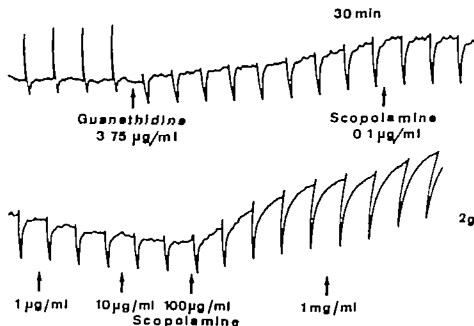


Fig 45 Penile artery branch to ccp. Pretreated with barium chloride (50 μg/ml). Stim. 5 Hz for 5 sec at 5 min interv Guanethidine blocks the excitatory response and uncovers the inhibitory response. *Cf* Figs. 17 and 27 Scopolamine in increasing concentrations does not suppress the inhibitory response. High concentrations of scopolamine increase the tone of the strip. Lower panel is a continuous record from the upper curve.

Cocaine (100 ng/ml) enhanced the excitatory response to nerve stimulation and increased the tone of the muscle strips. No clear-cut effect on the inhibitory response was observed.

Parasympathomimetic and antimuscarinic agents

As in the retractor penis muscle three different effects of *acetylcholine* were seen in the arterial strips 1) acetylcholine (0 01—100 μg/ml) contracted the strips in a dose-dependent way (Figs. 46a and b). The sensitivity to acetylcholine varied considerably between preparations from different bulls but no clear difference was observed between various segments of the penile artery from the same bull. This effect of acetylcholine was blocked by 1—10 μg/ml of

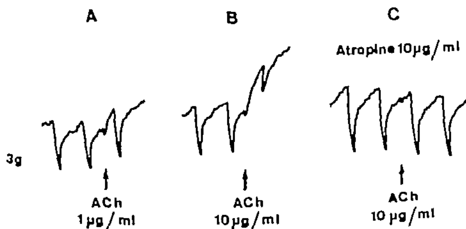


Fig. 46. Penile artery branch to ccu. Spontaneously in high tone. Stim. 2 Hz for 10 sec at 5 min interv. A. Moderate contraction to 1 µg/ml of acetylcholine. B. Stronger contraction to 10 µg/ml of acetylcholine. The reduction in size of the inhibitory response is probably due to physiological antagonism. C. After atropinization for 20 min, acetylcholine is without effect. Note also that atropine does not reduce the inhibitory response to field stimulation. *Cf* Figs. 22 and 47

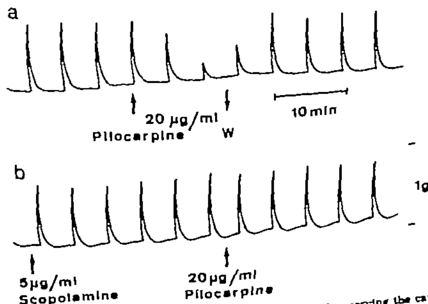


Fig. 47 Penile artery segment of the stem between the branches serving the cavernous bodies Stim. 6 Hz for 10 sec at 5 min interv. a. *Pilocarpine*, in a concentration having almost no effect on the tone of the strip, suppresses the excitatory response to field stimulation. W = washing. b. After scopolamine pilocarpine has no effect on the excitatory response. Note also that when the strip is slightly contracted signs of an inhibitory response following the excitatory response become visible. *Cf* Figs. 22 and 46.

Adrenergic neuron blocking agents and other compounds influencing adrenergic mechanisms

Bretylium (3—12 μg/ml) and *guanethidine* (0 7—4 μg/ml) blocked the excitatory response to nerve stimulation (Fig. 45) Both agents sometimes increased the tone of the arterial preparations but this effect was never as pronounced as in the retractor penis strips. Neither of them seemed to directly affect the inhibitory response to field stimulation, but they uncovered it as a consequence of the suppression of the excitatory response.

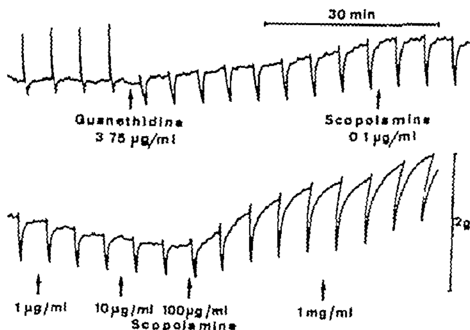


Fig. 45 Penile artery branch to ccp. Pretreated with barium chloride (50 μg/ml). Stim. 5 Hz for 5 sec at 5 min interv Guanethidine blocks the excitatory response and uncovers the inhibitory response. Cf Figs. 17 and 27 Scopolamine in increasing con- [illegible] does not suppress the inhibitory response. High concentrations of scopolamine increase the tone of the strip. Lower panel is a continuous record from the upper curve.

Cocaine (100 ng ml) enhanced the excitatory response to nerve stimulation and increased the tone of the muscle strips. No clear-cut effect on the inhibitory response was observed.

Parasympathomimetic and antimuscarinic agents

As in the retractor penis muscle, three different effects of *acetylcholine* were seen in the arterial strips 1) acetylcholine (0 01—100 μg ml) contracted the strips in a dose-dependent way (Figs 46a and b) The sensitivity to acetylcholine varied considerably between preparations from different bulls but no clear difference was observed between various segments of the penile artery from the same bull. This effect of acetylcholine was blocked by 1—10 μg/ml of

DMPP (0 6—60 μg/ml) contracted the arterial preparations. This contraction was not inhibited by phentolamine (2 μg/ml). The excitatory response to nerve stimulation was blocked by 6—60 μg/ml of DMPP but the inhibitory response was unimpaired. TMA (5—50 μg/ml) produced a marked increase in the tone of the strips. As a consequence of this the inhibitory response brought about by nerve stimulation became greater.

Hexamethonium (0.1—2 mg/ml) produced a moderate but distinct contraction of the arterial strips. No definite effect was observed on either kind of response to nerve stimulation. The same applies to *mecamylamine* (10—100 μg/ml). TEA in concentrations of 0 6—60 μg/ml increased the tone of the strip and enhanced the excitatory response evoked by nerve stimulation. There was no overt effect on the inhibitory responses.

Neuromuscular blocking agents

d *Tubocurarine* (10—100 μg/ml) *succinylcholine* (8—80 μg/ml) and *decamethonium* (8—80 μg/ml) increased the tone of the arterial strips. The excitatory response to nerve stimulation was moderately increased. The inhibitory response became greater due to the increase in tone.

Agents interfering with the synthesis destruction or release of acetylcholine

When the effect of *hemicholinium* (80—400 μg/ml) was investigated the preparations were stimulated with 30 imp/sec for 10 or 20 sec at 2 or 3 min intervals during 3 to 5 hours. A moderate decrease in the size of the inhibitory response elicited by this intense stimulation was seen in most preparations during the course of the experiment. But an identical decrease was seen in untreated preparations subjected to the same stimulation pattern. However when the frequency was switched back to the usual 5—10 imp/sec no definite decrease in the inhibitory response could be observed after hemicholinium.

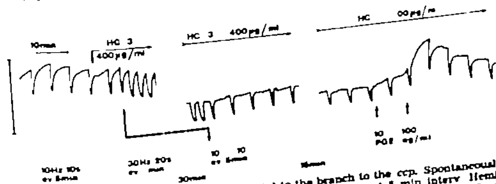


Fig. 49 Penil artery segment of the stem distal to the branch to the ccp. Spontaneously in high tone (about 1 4 g). *Left* pa el. stim. 10 Hz for 10 sec 1 5 min interv. Hemicholinium (400 μg/ml) is added to the bath and stimulation changed to 30 Hz for 20 sec 1 2 min interv. *Middl* pa el. after 4 h and 30 min stimulation is switched back to the original parameters. The tone of the strip has dropped, but as seen in the right pa[nel] no certain reduction of the inhibitory response to field stimulation can be noted when the tone is raised by prostaglandin E_2

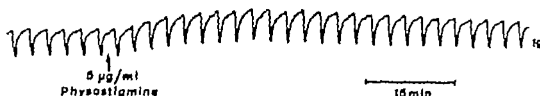


Fig 50. Penile artery branch to ccp. Spontaneously in tone. Stim. 5 Hz for 5 sec at 3 min interv Physostigmine increases the tone but exerts no certain influence on the *inhibitory response to field stimulation.*

provided the preparation was in the same stage of contraction as before application of the drug (Fig 49). In arterial strips it was not possible to examine the effect of hemicholinium on the excitatory responses evoked by field stimulation. This was due to the fact that excitatory responses evoked by 30 imp/sec faded considerably within 90 min whether the strips were exposed to hemicholinium or not. Hemicholinium by itself moderately relaxed the strips.

Physostigmine (1—5 µg/ml) increased the tone of the arterial preparations. This effect lasted for about 30 min, whereafter the tone slowly returned to the initial level. The magnitude of the inhibitory response was increased concomitantly with the increase in tone But when the preparation had regained its initial tone the size of the inhibitory response was the same as before the exposure to physostigmine (Fig 50). In relaxed preparations physostigmine produced a moderate increase in the excitatory responses to field stimulation.

Botulinum toxin was used in the concentrations of 4.5×10^3 to 1×10^7 MLD/ml. No distinct effect was seen on the inhibitory response to field stimulation in the course of 5 hours, which was the longest observation period used. Since the experiments were undertaken on strips treated with ergotamine (2—3 µg/ml) in order to secure a suitable tone no statement can be made concerning presumptive effects of the toxin on the excitatory response to field stimulation. The toxin preparation relaxed the artery when higher concentrations were added to the bath ($4.5 \times 10^6 - 1 \times 10^7$ MLD) but recovery of tone soon occurred and the initial tone was regained in about 30 min.

Histamine 5-hydroxytryptamine and agents blocking their receptors

Histamine (0 05—10 µg/ml) contracted the artery in a dose-dependent manner The contractions were abolished by mepyramine (0.1 µg/ml) phenbenzamine (0 1 µg/ml) and cyproheptadine (0 1—5 µg/ml) Also *5-hydroxytryptamine* (0 1—10 µg/ml) contracted the strips but these contractions exhibited considerable tachyphylaxis. They were inhibited by methysergide (0 1—5 µg) and cyproheptadine (1—5 µg/ml). Neither histamine nor 5-hydroxytryptamine seemed to have any direct influence on the inhibitory response elicited by nerve stimulation. The excitatory response was initially increased, *i.e.* before the preparation had gained a high tone *Mepyramine* (1—5 µg/ml) and *cyproheptadine* (1—5 µg/ml) relaxed the strips, whereas they were always contracted by *methysergide* (0 1—5 µg/ml) *Phenbenzamine* (0 1—2 µg/ml) had no overt effect on the tone. None of these receptor blocking compounds had any definite effect on the inhibitory response induced by nerve stimulation.

Prostaglandins prostaglandin antagonists and inhibitors of prostaglandin synthesis

$PGF_{2\alpha}$ (1—100 ng/ml), PGE_1 (0 01—1 μg/ml) and PGE_2 (0.01—1 μg/ml) all contracted the arterial strips in a dose-dependent way (*cf* Figs. 49 and 51). These contractions were counteracted by SC-19220 (10—20 μg/ml) and poly phloretin phosphate (1—10 μg/ml). The excitatory response to nerve stimulation was enhanced by all the three prostaglandins (*cf* Fig 51) The inhibitory responses increased in size when the preparations contracted (*cf* Figs. 49 and 51) SC 19220 (10—20 μg/ml) reduced the tone of the strips and the excitatory responses brought about by nerve stimulation. In part this was due to the propylene glycol in which the compound was dissolved. *Polyphloretin phosphate* (1—10 μg/ml) also relaxed the strips and suppressed the excitatory response produced by field stimulation. No clear effect was produced by the prostaglandin antagonists on the inhibitory response. *Indomethacin* (10—20 μg/ml) relaxed the arterial strips. Apparently this was mainly due to the phosphate buffer in which indomethacin was dissolved since the buffer alone also relaxed the strips. The inhibitory responses to nerve stimulation (10 imp/sec) were identical before and after three hours' exposure to indomethacin (10 μg/ml) during which the strips were stimulated with 30 imp/sec for 20 sec every 2 min. This statement is based on judgement of preparations in which the tone was raised with appropriate doses of histamine.



Fig. 51. Penile artery strip from the stem about 4 cm distal to the branch to the ccp Stim. 5 Hz for 5 sec at 5 min interv Prostaglandin E_1 increases the tone and the excitatory response to field stimulation The inhibitory response grows with the increase in tone. This strip was from the most distal segment of the penil artery used in the present investigation. Note also the slight rebound contraction following the inhibitory response. *Cf* Fig 30.

Amino acids

When studied in the concentrations of 1—10 μg/ml the following amino acids were without any definite effect on the arterial strips *GABA*, *glycine* *cysteine* *tryptophan*, *N-acetyltyrosine* *tyrosine*, *threonine* *phenylalanine*, *serine* *isoleuci e* *leucine* *valine* *alanine* *glutamic acid* *aspartic acid* *glutam ne* *asparagine* *histid ne* *arginine* *methionine* *proline* *hydroxyprol ne* and *lysine*.

Nucleosides and nucleotides

Adenosine (5—10 μg/ml) produced a moderate relaxation of the arterial strips. *Guanosine* (10 μg/ml), *cytidine* (10 μg/ml) and *inosine* (10 μg ml) were without any definite effect.

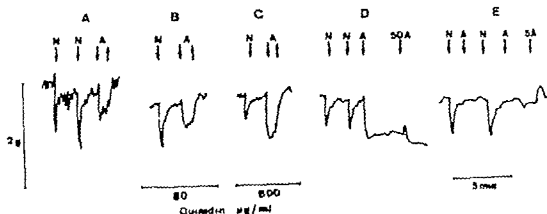


Fig. 52. Penile artery branch to ccp. Spontaneously in tone. At N transmural nerve stimulation with 2 Hz for 5 sec. At A ATP 10 μg/ml.
A. Control record showing relaxations to transmural nerve stimulation and ATP. B. In the presence of quinidine, 50 μg/ml for 10 min, the response to nerve stimulation is reduced but not the response to ATP. C. After quinidine, 500 μg/ml for 15 min, the response to nerve stimulation is further reduced, while the response to ATP is increased. D. 90 min after washing out quinidine the responses to nerve stimulation are still reduced and the response to ATP is increased. ATP is not washed out and 500 μg/ml of ATP is added at 50A. This high ATP concentration is left in the bath for 30 min. E. After repeated washings the preparation has gained tone. The responses to nerve stimulation are increased, possibly due to recovery from quinidine, but the preparation is now insensitive to 10 μg/ml of ATP. However, 50 μg/ml of ATP (at 5A) contracts the strip. Note also the spontaneous movements in the control record. They are suppressed by quinidine although the drug has no certain effect on the tone of the strip.

ATP (1—10 μg/ml) regularly produced a relaxation of the strip (Fig. 52 a), sometimes preceded by a brisk contraction. The relaxation was not counteracted but rather enhanced by quinidine in concentrations (5—500 μg/ml) which suppressed the responses to nerve stimulation (Figs. 52 b and c). However it was easy to desensitize the strips to the relaxing effect of low concentrations of ATP by exposing them to high concentrations of ATP (0.5—1 mg/ml) for 30 min (Figs. 52 d and e). After this treatment low concentrations of ATP had no or even a reversed, effect but the inhibitory response to nerve stimulation was unimpaired (Fig. 52 e). *ADP* (10 μg/ml) and *AMP* (10 μg/ml) also produced relaxations but they were considerably smaller than those brought about by ATP.

Cyclic AMP, CTP, GTP, GDP, GMP and *ITP* all in 10 μg/ml, had no definite effects on the penile artery preparations.

Peptides

No clear-cut effect of *bradykinin* or *eledoisin* was seen until the concentration had reached 0.1—2 μg/ml. Then the strips relaxed. But this was probably due to the solvent of the peptide preparations since identical relaxations were produced by corresponding concentrations of the solvents alone. *Substance P* (0.1—1 μg/ml) slightly contracted the arterial strips. Neither the excitatory nor the inhibitory response to field stimulation seemed to be directly affected by these peptides.

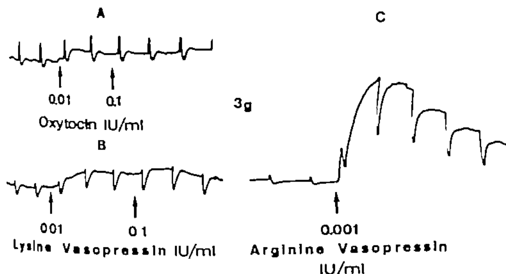


Fig. 53. Penile artery. Three different strips from the same bull. In *A* branch to ccp, *B* and *C* branches to ccs. Stim. 6 Hz for 5 sec t 5 min interv *A*. Moderate contraction by oxytocin. *B*. Contraction by lysine vasopressin. *C* Strong contraction by arginine vasopressin. Note the marked tachyphylaxis to the hormones shown in *A* and *B* and the considerably stronger effect of arginine vasopressin compared with the other hormones. *C* also illustrates the uncovering of the inhibitory response to field stimulation brought about by a contracting substance. *Cf* Fig. 37

Oxytocin (0.01—1 IU/ml) *lysine vasopressin* (0 01—1 IU/ml) and especially *arginine vasopressin* (0 001—0 1 IU/ml) contracted the arterial preparations (Fig 53). The effects of the posterior pituitary hormones exhibited considerable tachyphylaxis. None of these hormones seemed directly to affect the responses to nerve stimulation.

Angiotensin amide (0 05—1 μg/ml) contracted the strips, slightly enhanced the excitatory response, and unmasked the inhibitory response to nerve stimulation. The effects of also angiotensin exhibited considerable tachyphylaxis.

Pancreatic kallikrein (0 02—0.2 biological units/ml, Padutin®) raised the tone of the penile artery and induced rhythmic activity in the preparation. *Aprotinin* substance (1—200 kallikrein inactivating units/ml) on the other hand, relaxed the strip

Miscellaneous compounds

Barium chloride (10—100 μg/ml) raised tone of the strips (Figs. 41 and 43) facilitated the excitatory as well as the inhibitory response to nerve stimulation (Fig. 43) and triggered spontaneous activity *Ergotamine* (1—5 μg/ml) produced a conspicuous contraction of the artery and exposed the inhibitory response evoked by nerve stimulation (Fig. 54)

The arterial strips were markedly relaxed by *papaverine* (0 1—1 g/ml) and *theophylline amine* (0.8—8 μg/ml) These drugs also depressed the excitatory response to field stimulation. *Quinidine* (8—800 μg/ml) attenuated both types of response to field stimulation, but particularly the excitatory one. The drug had no unmistakable effect on the tone of the strips but depressed spontaneous rhythmic activity if present. *Strychnine* (10 μg/ml) and *picrotoxin* (10 μg/ml)

β Adrenoceptors

The present study further confirms the existence of adrenergic β-receptors in the retractor penis (Klinge 1970c) and the penile artery (Penttilä 1966) of the bull. Beta-receptors may also exist in the dog retractor penis (Edmunds 1920 Luduena *et al.* 1949). The physiological significance of the inhibitory β-effect remains obscure particularly as the motor α-effect is so dominant. The present studies with adrenergic blocking agents provide no evidence for any appreciable activation of β-receptors at field stimulation of the intramural nerves. In this connection it should be mentioned that we paid particular attention to the possibility that β-adrenoceptor activation would lead to an increased frequency of spontaneous contractions, i.e. β-receptor activation would have a positive chronotropic effect on the retractor penis and the penile artery as reported for the portal vein (Johansson *et al.* 1967). We never saw any effect of this kind. Isoprenaline always inhibited spontaneous activity. This does, however not disprove that there could be a positive β-effect on pacemaker activity in the retractor penis and the penile artery. Since β-adrenoceptor activation probably implies hyperpolarization of the smooth muscle cell membranes (*cf. e.g.* Sjöstrand 1973b) it is quite possible that a reduced ability to propagate action potentials would mask any increased activity from pacemaker areas. Particularly if the smooth muscle of the bull retractor penis and penile artery have the same low conduction velocity and ability as the dog retractor penis (Prosser Burnstock and Kahn 1960 Burnstock and Prosser 1960). The possibility therefore remains that locally in pacemaker areas β-adrenoceptor activation could increase the discharge frequency. Finally it is of course not at all necessary to ascribe any physiological significance to β receptors present in organs as the retractor penis and the vas deferens.

Some quaternary ammonium compounds

The close recemblance of some of the effects of DMPP to those of guanethidine and bretylium is in accordance with the concept that this drug also possesses adrenergic neuron blocking properties (Bentley 1962 Birmingham and Wilson 1965 Nedergaard and Bevan 1969).

The rise in tone brought about in the retractor penis and the penile artery by various quaternary ammonium compounds as hexamethonium, TMA and decamethonium may rather be due to a direct muscular effect than to release of *e.g.* noradrenaline. This assumption is favoured by the fact that their contracting effect was greater on the penile artery than on the retractor penis in contrast to that of bretylium and guanethidine. The TEA induced enhancement of the excitatory response to nerve stimulation is probably due to a facilitated release of the adrenergic neurotransmitter (Thoenen *et al.* 1967 Kirpekar *et al.* 1972)

Local anaesthetics

The increased tone of the retractor penis and the penile artery caused by the local anaesthetics is likely to be a direct smooth muscle effect and not due to release of noradrenaline for the same reasons as the effect of some of the quaternary ammonium compounds, i.e. the effect was usually greater on the

penile artery than on the retractor penis. In the case of procaine the contractile effect was seen only on the artery while the effect on the retractor penis was relaxation.

Hemicholinium botulinum toxin and morphine

Hemicholinium suppresses cholinergic neurotransmission presumably by interfering with the transport of choline to intraneuronal sites (MacIntosh et al. 1956). The onset of the block is known to depend on the concentration of hemicholinium, the frequency of stimuli and the interval between pulse trains (Reitzel and Long 1959) In order to check that the concentration, the time of exposure and the stimulation parameters used were sufficient the following experiments were done. Coaxial stimulation (Paton 1955) of the guinea-pig ileum with 30 imp/sec for 20 sec every 2 min and preganglionic stimulation (Sjöstrand 1962) of the guinea-pig vas deferens (Hukovic 1961) with 20 imp/sec for 10 sec every 2 min were performed in the presence of hemi cholinium (60 μg/ml) Atropine-susceptible responses of the ileum were almost abolished within one hour The same was the case with the responses to preganglionic stimulation of the vas deferens, whereas the responses to post ganglionic stimulation (Birmingham and Wilson 1963) were almost unaffected. In the retractor penis and the penile artery of the bull no definite reduction of the inhibitory or the excitatory response to field stimulation was observed when preparations exposed to hemicholinium were compared with identically stimulated control strips.

Botulinum toxin type A is regarded to be a specific inhibitor of acetylcholine release from various cholinergic nerve endings (Ambache 1949 Burgen et al. 1949 Ambache 1951) However it has been reported that the toxin can depress adrenergic transmission too although the adrenergic nerve terminals are much more resistant to it (Holman and Spitzer 1973). Thus the reduction in size of the excitatory response seen in this study after botulinum toxin may be due to an impaired release of the transmitter substance from the adrenergic nerve terminals. If this is the case, then the present results indicate that in the retractor penis and the penile artery of the bull the terminals of the inhibitory nerves are still more resistant to the toxin than those of the adrenergic nerves. The efficacy of the botulinum toxin used was tested on the coaxially stimulated guinea-pig ileum and it was found that 4.5×10^4 MLD (mouse)/ml almost abolished atropine-susceptible responses to nerve stimulation within 45 min.

Morphine was included in the present study because of its inhibitory action on acetylcholine output from cholinergic nerves in the intestine (Schaumann 1957 Paton 1957). Morphine has also been shown to depress the response to adrenergic nerve stimulation in some organs (Szerb 1961 Cairnie et al. 1961) but not in all (Cairnie et al. 1961). In the present study no effect was seen either on the inhibitory or on the excitatory response to field stimulation.

Prostaglandins and prostaglandin receptors

The possible role of the prostaglandins as neutrotransmitters will be discussed in the next chapter In this connection some other [redacted] will be briefly considered. The prostaglandins E_1 and E_2 have been [redacted] to suppress the release of noradrenaline induced by nerve stimulation [redacted] to diminish motor responses to adrenergic nerve stimulation ([redacted] and

Brundin 1969 Euler and Hedqvist 1969 for further references see Hedqvist 1973). Because the direct relaxing effect of PGE_1 on the bull retractor penis is so strong, it is impossible to state anything about a presumptive additional contribution of a diminished transmitter secretion when the reduction of the excitatory response to field stimulation after PGE_1 is concerned. The reduction of the excitatory response to nerve stimulation exhibited, however close correlation with the reduction of the motor response to exogenous smooth muscle stimulants.

Since in the penile artery contrary to the retractor penis, the motor response to nerve stimulation was enhanced by all the prostaglandins studied, they probably did not produce a considerable reduction in the release of noradrenaline in that preparation. Maybe the bovine adrenergic neurotransmission is not as sensitive to prostaglandins as *e.g* the feline adrenergic neurotransmission. The enhancement of the excitatory response to field stimulation is probably due to the direct stimulant effect of the prostaglandins on the smooth muscle of the artery (*cf* Sjöstrand 1972).

The prostaglandin antagonist SC-19220 suppressed the excitatory effects of the prostaglandins on the penile artery whereas it did not counteract the inhibitory effect of PGE_1 on the retractor penis. This is compatible with findings in other smooth muscles and further illustrates that prostaglandin receptors mediating relaxation are different from those mediating contraction (Eakins and Sanner 1972)

Smooth muscle stimulants

The reduction in magnitude of the inhibitory response to nerve stimulation frequently seen at the onset of the contraction produced by various stimulating agents, as *e.g* barium or noradrenaline, and also when high concentrations of such agents are used, is likely to be due to physiological antagonism. An additive effect due to suppression of the inhibitory neurotransmission in the case of drugs as prostaglandins, α-adrenoceptor stimulants, and muscarinic agents which are known to affect autonomic adrenergic and cholinergic neurotransmission, can of course not be excluded.

The enhancement of the excitatory response often brought about by contracting agents is probably due to a direct synergistic effect on the smooth muscle (Sjöstrand and Swedin 1968 Sjöstrand 1973a).

The effects of barium require some further comments. The general effect of the ion was to raise the tone of the preparations and to facilitate the responses to nerve stimulation. It is known that barium depolarizes smooth muscle and also increases amplitude and duration of action potentials in smooth muscle (see Kuriyama 1970) Barium further facilitates the release of nor adrenaline from adrenergic nerve terminals (Kirpekar *et al.* 1972) Thus the enhancement of the excitatory response to nerve stimulation observed in the present preparations could be the resultant of both neuronal and smooth muscle actions. The facilitation of the inhibitory response to nerve stimulation might as well be the resultant of the raised tone and an increased release of the respective neurotransmitter

The brief relaxation of the retractor penis observed when the potassium concentration was doubled might be due to excitation of the inhibitory nerves since a more pronounced increase of potassium usually depolarizes smooth muscle and induces contraction.

Quinidine and ATP receptors

Quinidine was tested because this drug has been reported to block the inhibitory effect of ATP on intestines (Bowman and Hall 1970 Burnstock *et al.* 1970) and the excitatory effect of ATP on the urinary bladder (Burnstock *et al.* 1972) and has therefore been used in the analysis of purinergic transmission (Burnstock 1972). In the present study quinidine slightly counteracted the excitatory effect of ATP on the retractor penis while it exerted no antagonistic action at all on the ATP induced relaxation of the penile artery Burnstock and coworkers (1972) found that quinidine reversed the excitatory effect of ATP on the urinary bladder of the quinea-pig Thus different receptors for ATP (quinidine-susceptible and not quinidine-susceptible) may occur in smooth muscle (Burnstock 1972). In the present study quinidine inhibited the responses to field stimulation in the retractor penis and the penile artery but in both tissues the excitatory response was considerably more susceptible to this effect than the inhibitory response. This effect of quinidine is probably to a great extent due to the localanaesthetic property of the drug which is characterized by rather long duration.

CHAPTER VI

DISCUSSION

The smooth muscle of the retractor penis and the penile artery

Obviously the bull retractor penis possesses certain properties characteristic of single unit smooth muscle (Bozler 1948) Thus, it can exhibit spontaneous rhythmic activity This indicates that at least part of the muscle cells have pacemaker activity The rhythmic contractions, as illustrated in e.g Fig. 5 have considerable amplitude and duration. This implies that a large muscle area is involved in the contraction and indicates a spread of the activation from cell to cell. Single unit characteristics have been demonstrated with electrophysiological methods in the dog retractor penis (Orlov 1963a) The early findings of Sertoli (1883) De Zilwa (1901) and Edmunds (1920) also point in this direction. The frequency of rhythmic contractions is low Usually 1 to 3 minutes elapsed between the peaks of each contraction (e.g Figs. 5 and 6) To some extent this could have been due to the low temperature (35 C) in the organ bath. But this temperature is probably a rather physiological temperature with respect to the extraabdominal parts of the muscle. A rather low conduction velocity of the muscle due to structural arrangement and fibre size of the smooth muscle cells may also contribute to the low frequency of spontaneous contractions. This seems to be the case for the dog retractor penis (Prosser Brunstock and Kahn 1960 Burnstock and Prosser 1960) The present statement concerning the smooth muscle of the retractor penis which is based on *in vitro* experiments seems to be valid also in *in vivo* conditions. Recently Ashdown and Pearson (1973) observed during surgery on anaesthetized bulls that the tonus of the muscle is not uniform troughout the length of the muscle. Contraction seems to occur at irregular intervals along the length of the muscle at these points the muscle shortens Presumably also the smooth muscle of the penile artery possesses certain single unit properties as indicated by the spontaneous activity exhibited by several preparations. However the spiral cutting probably deteriorates the muscle. Therefore at present a definite statement is not possible.

Excitatory nerves and motor responses to field stimulation

The excitatory nerves in the bull retractor penis and penile artery activated by field stimulation are apparently adrenergic and the receptors mediating excitation α-adrenoceptors. The contractions evoked by transmural stimulation are abolished or profoundly depressed by adrenergic neuron and α-adrenoceptor

blocking agents and augmented by appropriate concentrations of inhibitors of neuronal noradrenaline uptake. Further the indirectly acting sympathomimetic amine tyramine contracts the retractor penis. Contractions elicited by exogenous noradrenaline are also enhanced by compounds known to inhibit the neuronal uptake of this agonist. Finally the organs have a fairly dense adrenergic innervation (Klinge, Pohto and Solatunturi 1970 Klinge and Pohto 1971) and the retractor penis contains large amounts of the adrenergic neurotransmitter (Klinge 1970a). The reviewed literature (Chapter I) indicates that most of the adrenergic fibres reach the retractor penis and the penile artery via the pudic nerve, but a small portion may run down along the hypogastric or the pelvic nerve.

In this connection it should be noted that the bull retractor penis is highly sensitive to exogenous noradrenaline. This is often not the case with organs having a dense adrenergic innervation, as *e.g* the vas deferens (Sjöstrand 1961 1965). High sensitivity to the excitatory transmitter in a densely innervated smooth muscle is obviously an advantage if the organ is intended to be kept in continuous tone, as the retractor penis. Hence *in vivo* the tonic discharge of the excitatory nerves can be very low particularly as the smooth muscle itself exhibits spontaneous activity The possibility that *in vivo* the bull retractor penis is maintained in continuous contractile activity by a very low impulse frequency in the adrenergic nerves is illustrated by the present *in vitro* results strong contractions could be produced by single pulses (Figs. 8 and 22) and continuous stimulation at very low frequency (0 1 imp/sec) could keep spontaneously active strips in tone (Fig 5)

The excitatory frequency response curve to 10 sec stimulation of the retractor penis obtained in this study is however fairly flat (Fig 9a) To a great extent this is certainly due to the counteracting inhibitory response simultaneously elicited by field stimulation as indicated by the curve presented in Fig. 9b. But it should also be emphasized that even if the maximum excitatory response *in vivo* would require a rather high frequency *e.g* 10—15 imp/sec this is not incompatible with the assumption that a low tonic discharge is capable of maintaining the muscle in appropriate contraction. The tensions developed by the tiny retractor penis strips, when stimulated with 16—32 imp/sec, were indeed impressive, i.e. they often exceeded 40 grams. Thus, the feasible maximum force of the whole muscle must be considerable and far exceeds the tension necessary to keep the penis withdrawn in the prepuce.

The excitatory frequency response curve of the penile artery presented in this report may with due reservation for the fact that it is obtained from helically cut and thus injured strips, give certain information on the arterial smooth muscle as a neuro-effector system. However *in vivo* the relevant matter is the resistance to blood flow of the arteries which apart from changes in blood viscosity type of flow and length of the tube varies with the reciprocal of the fourth power of the radius of the vessel. Naturally it is impossible to correctly transfer the present frequency response curve of relative isometric tension of arterial smooth muscle to an *in vivo* frequency response curve of vascular resistance to blood flow But such a curve would most likely have its maximum at a much lower frequency than the curve presented in this report. Probably a very low vasomotor tone is needed to keep a high resistance in the arteries supplying the cavernous bodies, as is the case in most vascular beds (Folkow 1952, Mellander 1960 Folkow and Neil 1971).

Inhibitory nerves and relaxations induced by field stimulation

The inhibitory response to field stimulation of the retractor penis and the penile artery is obviously of neural origin since it is abolished by tetrodotoxin and local anaesthetics. From the information given by the reviewed literature it seems reasonable to assume that the inhibitory nerves excited by transmural stimulation are mainly postganglionic parasympathetic fibres, although it can not be excluded that some fibres could belong to the sympathetic nervous system. Further from evidence in the literature as well as in the present study it seems reasonable to assume that the inhibitory nerves in the retractor penis and those in the penile artery are of the same kind and that they act by releasing an inhibitory agent.

Our results support in no way the concept that the neurotransmitter which relaxes the smooth muscle responsible for erection is acetylcholine. On the contrary the data speaking against acetylcholine as the inhibitory transmitter of these muscles are numerous. Acetylcholine and pilocarpine contract the retractor penis as well as the penile artery (the relaxations sometimes produced by acetylcholine or nicotine will be discussed below). These contractions are inhibited by atropine and scopolamine. Thus, in the strips of retractor penis and penile artery examined in the present study the muscarinic receptors accessible to exogenous cholinomimetics are excitatory With reservation for the contraction due to physostigmine it can be stated that this drug does not enhance the inhibitory response to field stimulation. Nor is this response impaired by even extreme concentrations of atropine or scopolamine. The response is not inhibited by ganglionic or neuromuscular blocking agents. This as well as the short lasting and tachyphylactic effect of ganglionic stimulants (see below) makes it unlikely that the smooth muscle cells have inhibitory nicotinic receptors. The vigorous contractions of the retractor penis induced by even minute concentrations of bradykinin and the uncertain effect of this compound on the penile artery as well as the observed effects of kallikrein and aprotinin make it unlikely that acetylcholine could relax the muscles indirectly via kinin release Finally and most conclusively the inhibitory response is unimpaired by hemicholinium and botulinum toxin. For the above reasons we conclude that the inhibitory responses to field stimulation are not due to release of acetylcholine from inhibitory nerves. In this respect we agree with Luduena and Grigas (1966).

Luduena and Grigas (1972) have suggested that the inhibitory neurotransmission could be prostaglandinergic. We find this unlikely It is true that PGE_1 relaxes the retractor penis of the bull as it relaxes the retractor penis of the dog (Luduena and Grigas 1972) and the cat (Klinge and Sjöstrand, unpublished) However the inhibitory responses to field stimulation are uninfluenced by inhibitors of prostaglandin synthesis. Further the three common prostaglandins PGE_1 PGE_2 and $PGF_{2\alpha}$ contract the penile artery This is a strong argument against at least these prostaglandins as the inhibitory neurotransmitter

It is also unlikely that the inhibitory neurotransmission of the retractor penis and the penile artery is purinergic. ATP ADP and AMP contract the retractor penis. The penile artery is relaxed by ATP but this effect can easily be abolished or even reversed by desensitization to ATP However this treatment has no effect on the inhibitory response to field stimulation. The other nucleotides and nucleosides studied were without any distinct effect, but this could have been due to penetration difficulties.

The present study provides no evidence in favour of histamine, 5-hydroxytryptamine or Substance P as the inhibitory neurotransmitter since all these compounds contract both the retractor penis and the penile artery. Further the inhibitory response to field stimulation is uninfluenced by antihistamines and 5-hydroxytryptamine antagonists. It is also unlikely that GABA, glycine aspartic acid, glutamic acid or any other of the amino acids studied would be the inhibitory transmitter. As a matter of fact the present study does not give even a hint of the nature of the inhibitory neurotransmitter in the retractor penis and the penile artery of the bull.

Recently Gillespie and coworkers (Gillespie 1972, Gibson and Gillespie 1973, Gillespie and McGrath 1973) have studied the rat anococcygeus muscle as well as that of the cat (Gillespie and McGrath 1974). This muscle exhibits great similarities with the retractor penis with respect to e.g. the responses to transmural nerve stimulation and the reaction to adrenergic neuron blocking agents. This is hardly a coincidence. In the bull (Heinemann 1937) as well as in the dog (Langley and Anderson 1895) the anococcygeus muscle has a common origin with the retractor penis. Presumably the two muscles have an identical nervous supply. Of particular interest are the findings that treatment with 6-hydroxydopamine or immunosympathectomy does not interfere with the inhibitory response to field stimulation of the rat anococcygeus (Gibson and Gillespie 1973). Further the excitatory adrenergic innervation and the inhibitory innervation of the rat anococcygeus have different spinal origins (Gillespie and McGrath 1973). Therefore, the inhibitory and the excitatory nerves must constitute separate entities.

Although the nature of the inhibitory neurotransmitter remains unsettled, the present study gives certain information on the responses of the retractor penis and the penile artery to inhibitory nerve stimulation. A characteristic feature of the inhibitory response of the retractor penis is the very steep frequency response curve and the easily obtained maximum inhibition. Since additional relaxation could be obtained by e.g. papaverine the restriction of the inhibition induced by nerve stimulation can not be due to a limited capacity of relaxation. Since shortening of the duration of the impulse train and a decrease in stimulation voltage (i.e. decreased number of activated nerve fibres) both shifted the frequency-response curve to the right, i.e. towards higher frequencies, without changing the degree of maximum inhibition, and further since the inhibitory response was mainly dependent on the number of impulses and not on their frequency it is hardly a limited capacity of transmitter secretion from the nerves which restricts the inhibitory response. This is also evident when the frequency response curve of the retractor penis and that of the penile artery are compared. Thus, since it obviously is neither the capacity of relaxation of the smooth muscle nor the capacity of transmitter secretion of the inhibitory nerves which is responsible for the limited maximum inhibition of the retractor penis, it must be an event between transmitter release and relaxation which restricts the inhibition produced by nerve stimulation. A plausible explanation is that the number of receptors for the inhibitory transmitter is relatively limited and that a high degree of receptor occupancy is quickly established. An alternative explanation would be that one of the reactions following occupation of the receptors has rapidly reached maximum, e.g. the equilibrium potential of the inhibitory transmitter may be promptly achieved. The rapid and reproducible relaxations in response to nerve stimulation as well as the almost instantaneous cessation of the relaxation when stimulation is interrupted indicate that the sites of transmitter

release and elimination are located close to the receptors in the retractor penis. Probably the retractor penis has a rather dense inhibitory innervation. The high dependence of the inhibitory response on the number of impulses, regardless of the frequency at which they are applied, suggests that the secretion of the inhibitory transmitter shows little facilitation when the interval between the pulses is shortened With reservation for the fact that the arterial preparation of this study certainly is rather injured, it seems that it has not as intimate a relationship between the inhibitory nerves and the receptors as the retractor penis.

Further speculations on the nature and the receptors of the transmitter of the inhibitory nerves would be premature The functional implications of the present findings may be considered, however As an autonomic neuro-effector unit the bull retractor penis with its inhibitory nerves must be regarded as a very efficient system. In almost every preparation a total number of 80 impulses delivered at frequencies ranging from 0.5 to 10 Hz produced maximum inhibition. Usually 10—20 impulses yielded 80—90 % inhibition, and 50 % inhibition or more was often obtained with only 5 impulses. This implies that *in vivo* the retractor penis could relax instantaneously on even moderate discharges in the inhibitory nerves.

The inhibitory frequency response relationship of the arterial strip was very similar to that of the excitation. Possibly *in vivo* the frequency response relationship between the inhibitory discharge and the vascular resistance would also be an almost reversed image of the corresponding excitatory relationship when dilation of constricted arteries is considered. The fundamental point, as evident from the reviewed literature, is that penile erection can not be brought about solely by interruption of the adrenergic vasomotor tone. Vasodilator innervation of the resistance vessels is apparently needed in this vascular bed. An obvious reason for this would be that, at least in the bull (see the anatomical outline) there is hardly any possibility for formation and action of local vasodilator metabolites as, *e.g.*, in muscular and glandular tissues.

The rebound contraction

The often long lasting rebound contraction observed in many preparations from the retractor penis when stimulation of the inhibitory nerves was terminated, is likely to be a true rebound effect similar to that described for the gut (Furness 1970 1971) The marked dependence of the rebound contraction on the degree of the inhibitory stimulation and on the tone of the muscle makes it unlikely that this secondary contraction (Furness 1971) is due to a transmitter release from a third type of nerve. Since moderate concentrations of phentolamine slightly diminished the rebound contraction and cocaine enhanced it, noradrenaline remaining at the receptor sites may to a small extent contribute to the size of the contraction. Rebound contraction of the retractor penis may be of physiological significance by facilitating a rapid termination of penile erection.

Acetylcholine and the role of possible cholinergic innervation

As stated above, it is unlikely that acetylcholine is the inhibitory transmitter responsible for the relaxation caused by transmural nerve stimulation.

However acetylcholine is present in the bull retractor penis in concentrations corresponding to those of the bovine duodenum and urinary bladder and the tissue also contains acetylcholinesterase (Klinge 1970b). Histochemical studies have shown that the acetylcholinesterase activity is located in nerves scattered in the muscle (Klinge, Pohto and Solatunturi 1970). These authors found no full correspondence between the location of the cholinesterase-positive fibres and the adrenergic fibres. In the dog retractor penis Bell and McLean (1970) found a rich distribution of adrenegic terminals along the whole length of the smooth muscle, while acetylcholinestorase-positive fibres were mainly located in the anterior part of the muscle. These authors concluded that the dog retractor penis probably was supplied by separate adrenergic and cholinergic nerves. Accordingly in the retractor penis of the bull, the cholinesterase-positive nerves may at least in part be separate from the adrenergic nerves. It is commonly assumed that acetylcholine present in tissues possessing cholinesterase-positive nerves is located in these nerves. If the above assumptions are valid the bull retractor penis would, in addition to adrenergic nerves, also be supplied by cholinergic nerves, parasympathetic or maybe sympathetic in origin. We will therefore discuss the observed effects of acetylcholine and of other muscarinic and nicotinic drugs on the presumption that the bull retractor penis is innervated by separate cholinergic nerves and we will consider the possible function of such presumptive nerves.

Two different muscarinic actions were observed 1) Contraction of the smooth muscle, and 2) suppression of the excitatory response to field stimulation. Presumptive cholinergic nerves might therefore constitute a component of the excitatory innervation of the muscle and/or contribute to its relaxation by suppressing adrenergic neurotransmission. The first alternative appears less probable although it can not be excluded. The pharmacological analysis of the excitatory response to nerve stimulation provides no clear substantial evidence for an appreciable cholinergic component in this response. Further many preparations were virtually insensitive to the contracting effect of high concentrations of acetylcholine. Low concentrations, i.e. less than 1 g/ml, rarely contracted the strips. The second alternative seems more probable. In some preparations the sole effect of pilocarpine or acetylcholine was suppression of the excitatory response to field stimulation. Inhibition of release f the adrenergic transmitter by muscarinic agents or by stimulation of cholinergic nerves has recently been demonstrated in many organs (e.g. Lindmar, Loffelholz and Muscholl 1968, Löffelholz and Muscholl 1970, Malik and Ling 1969, Vanhoutte and Shepherd 1973). Furthermore, peripheral adrenergic and cholinergic axons have been found to run concomitantly and tightly intertwined in several tissues (Eränkö and Raisanen 1965, Ehinger and Falck 1965, Jacobowitz and Koelle 1965, Tranzer and Thoenen 1967, Ehinger, Falck and Sporrong 1970). Finally the possibility should be k pt in mind that th cholinesterase-positive nerves are the fibres secreting the inhibitory transmitter perhaps together with acetylcholine alth ugh the transmitter could be secreted from a third kind of fibre as well.

The relaxing effect seen with nicotine and DMPP and sometimes with high concentrations of acetylcholin is probably due to stimulation of nicotinic receptors in the inhibitory nerves. This assumption is based on the finding that the effect is blocked by ganglionic blocking agents as well as by lidocaine. The point of ttack of nicotine is postganglionic rather than ganglionic since no autonomic ganglia have been observed in the retractor penis of several species (Fletcher 1897, Fischer 1917, Klinge, Pohto and Solatunturi 1970). This wo

be in accordance with earlier findings showing that nicotinic drugs activate pre- and postganglionic C fibres (*e g* Armett and Ritchie 1961 Schaeppi, Dennison and Dodd 1966) In analogy the small and rapid contraction sometimes preceding the inhibition may be due to nicotinic activation of excitatory adrenergic nerve fibres, and this could also apply to the contractions of the dog retractor penis brought about by nicotine in the studies of Edmunds (1920) and Luduena and Grigas (1966) The relaxations of the dog retractor penis produced by large concentrations of acetylcholine in the studies of Orlov (1963 b) and Gushchin (1965) were presumably due to nicotinic activation of the inhibitory nerves.

In this study any attempt to evaluate the validity of the original Burn and Rand hypothesis has deliberately been omitted. Accordingly we are unable to draw any conclusions although some findings might provide indirect evidence in favour of the theory as for instance the effects of botulinum toxin and physostigmine Alternative explanations as direct or indirect postjunctional effects of physostigmine and a direct effect of high concentrations of botulinum toxin on adrenergic neurotransmission (Holman and Spitzer 1973) are, however as possible. Acetylcholinesterase activity has been demonstrated in sympathetic adrenergic axons (Eränkö *et al.* 1970) So far the possible existence of acetylcholinesterase-positive acetylcholine-containing adrenergic fibres in the bull retractor penis can not be excluded. But if this is true then the question is, why are not all adrenergic fibres cholinesterase-positive?

Posterior pituitary hormones

Earlier findings have suggested that posterior pituitary hormones relax the bull and dog retractor penis (Klinge 1970 c, Luduena and Grigas 1972) and dilate the pudendal artery and its branches (Holmquist and Olin 1969) In the cited studies the hormones were stored in organic solvents. The present results indicate that the solvents alone were responsible for the relaxations observed. [illegible] the mere hormones had no effect on the retractor penis and contracted [illegible] penile artery it is unlikely that posterior pituitary hormones locally contribute to the establishment of penile erection. The considerably stronger vasoconstrictor effect of the bovine arginine-vasopressin in comparison with the porcine lysine-vasopressin further supports the above view

Some final remarks

The exact vascular events in penile erection of the bull are poorly understood. The possible importance of vasocompression due to activity of the ischiocavernosus muscle has been stressed by Watson (1964) The enormous peak pressure, 1727 mm Hg, in the corpus cavernosum penis during erection reported by Lewis *et al.* (1968) indicates that erection in the bull may not just be a simple matter of difference between in and outflow resistance of the cavernous bodies. Provided this value gives a correct picture of the pressure within the thick and fibrous tunica albuginea an active vasocompression must take place. The theory of active vasocompression as well as the correctness of the pressure values reported for the corpus cavernosum penis of the bull are indirectly supported by the huge pressures recently recorded during erection in the corpus cavernosum penis of the goat and the stallion (Beckett *et al.*

1972 a, b and 1973). Such pressure could be possible only in species in which the penis is covered by a specially thick tunica albuginea. The recent electromyographic studies of Hart (1972) indicate that also in the dog adjacent striated muscles play a role in the establishment of complete erection. An active compression effectuated by the adjacent striated muscles does, however not exclude that dilation of the inflow resistance vessels of the cavernous bodies is a prerequisite for full penile erection. In the termination of erection and reestablishment of the sigmoid flexure of the bull penis the structural architecture of the fibrous elements are probably of importance (Ashdown et al. 1968, Ashdown and Smith 1969).

The bull retractor penis is a smooth muscle which obviously can be kept in tone by a few excitatory nerve impulses but which also is promptly relaxed by a limited number of inhibitory impulses. The mere cessation of the inhibitory discharge may lead to replacement of relaxation by a strong rebound contraction. Thus the muscle is well adapted for its task, i.e. to permit an almost instantaneous protrusion of the penis during copulation and to produce a rapid withdrawal of the penis after copulation (*cf* Ashdown and Pearson 1973). In contrast to its slow endogenous activity the smooth muscle is very efficient as a neuroeffector unit. Previously it has been pointed out that the effectors of seminal emission, i.e. the smooth muscle of the vas deferens, seminal vesicle and prostate with their abundant adrenergic supply are well adapted for brief and forceful contractions (Sjöstrand 1965) thus facilitating rapid ejaculation (*cf* Seidel and Foote 1969) In the case of the smooth muscle effectors of erection the autonomic neuroeffector units are constructed for prompt relaxations as well. The physiological significance of this is that the time of copulation can be short. With some exceptions, as *e.g* domestic hog and also dogs, most mammals have very short copulation times. For animals of prey as *e.g* the bovidae, a short copulation time may be of advantage with respect to the exposure to predators. Among other things reproduction and reproductive behaviour involves an increased risk for capture of the animal.

Finally the validity of the present results for species other than ox should be considered. From the reviewed literature (Chapter I) it seems likely that the smooth muscle effectors of erection in other species also have an excitatory adrenergic innervation. Although under disputable experimental conditions, penile erection has been reported to be atropine-fast in boar dog cat, rabbit and also in guinea-pig It is thus possible that at least in these species erection may in part be effectuated by a non-cholinergic neurotransmitter Presumptive cholinergic fibres may however participate directly in the smooth muscle relaxation of erection in some species. Thus cholinesterase-positive fibres are present in the smooth muscle of the cavernous bodies of the rabbit and this muscle is relaxed by acetylcholine (*cf* Chapter I) It can of course also not be excluded that in certain species presumptive cholinergic fibres could be motor fibres.

SUMMARY

The effects of field stimulation and various endogenous compounds and drugs affecting the functions of autonomic nerves or receptors were investigated on isolated strips of the retractor penis muscle and the penile artery of the bull.
The following main results were obtained:

1) The retractor penis exhibited rhythmic spontaneous activity. Spontaneous activity was also seen in some penile artery preparations. Sustained tonic contractions were frequent in both smooth muscles.

2) Field stimulation of the smooth muscles induced a monophasic, biphasic or triphasic response. If the strips were in low tone a motor response dominated or was the only response observed. If the strips were in high tone a relaxation dominated or was the only response observed. In preparations being in medium stage of tone, the response to field stimulation was contraction followed by relaxation. In the retractor penis the relaxation was often followed by a secondary contraction. This was rare in penile artery strips.

3) The frequency response relationship of the motor response to field stimulation indicated a maximum response at high frequencies. However in the retractor penis strong contractions could be elicited by single shocks. Trains of stimuli delivered at low frequencies could maintain the muscle in sustained contraction.

4) In the penile artery the frequency response relationship of relaxation induced by field stimulation was similar to that of excitation. In the retractor [illegible]is the inhibitory response to field stimulation was characterized by a rather [illegible]ly delineated maximum occurring at a limited number of pulses.

5) The secondary contraction was mainly dependent on the grade of the inhibitory response to field stimulation. Its peak occured 2—3 min after cessation of stimulation.

6) The excitatory and the inhibitory responses to field stimulation as well as the secondary contraction were all abolished by tetrodotoxin or local anaesthetic drugs.

7) The excitatory response to field stimulation was inhibited or abolished by α-adrenoceptor and adrenergic neuron blocking agents. It was enhanced by inhibitors of neuronal noradrenaline uptake.

8) Noradrenaline and adrenaline contracted the retractor penis and the penile artery. This effect was abolished by α-adrenoceptor blocking agents. After α-receptor blockade adrenaline and noradrenaline produced relaxation. So did also isoprenaline. These relaxations were prevented by β-adrenoceptor blocking agents.

9) The inhibitory response to field stimulation was not prevented by antimuscarinic, ganglionic blocking or neuromuscular blocking drugs. Nor was it counteracted by botulinum toxin or hemicholinium. It also appeared to be unaffected by physostigmine. It was uncovered by adrenergic neuron blocking agents.

10) Three different effects of acetylcholine were noted. a) contraction of the smooth muscle, b) suppression of the excitatory response to field stimulation, and c) a brisk short lasting relaxation. This relaxation was sometimes preceded by a rapid contraction and resembled the effect of transmural nerve stimulation. The first two effects of acetylcholine were emulated by pilocarpine and prevented by antimuscarinic drugs. The third effect was prevented by hexamethonium and emulated by nicotine. Nicotine-induced relaxations were prevented by ganglionic blocking agents as well as by local anaesthetics. All three acetylcholine effects, particularly the last one, required high concentrations.

11) The retractor penis and the penile artery were contracted by histamine and 5-hydroxytryptamine. The inhibitory response to field stimulation was not blocked by antihistamines or serotonin antagonists.

12) The retractor penis was contracted by ATP The penile artery was relaxed by ATP This relaxation was abolished or reversed after »desensitization» to ATP This treatment had no effect on the inhibitory response to field stimulation. A number of other nucleotides and nucleosides were without effect or had weak effects similar to those of ATP

13) No overt effects on the retractor penis and the penile artery were obtained with GABA, glycine glutamic acid, aspartic acid or several other amino acids.

14) Prostaglandins E_1 and E_2 relaxed the retractor penis, whereas prostaglandin $F_{2\alpha}$ contracted it. All these prostaglandins were powerful stimulants of the arterial smooth muscle. Prolonged exposure to inhibitors of prostaglandin synthesis did not suppress the inhibitory responses to field stimulation.

15) Minute concentrations of bradykinin contracted the retractor penis. This peptide had almost no effect on the penile artery Substance P contracted both muscles. Posterior pituitary hormones had no overt effect on the retractor penis but contracted the penile artery

CONCLUSIONS

1) The smooth muscle of the retractor penis and probably also that of the penile artery of the bull possess certain characteristics of single unit smooth muscle.

2) The motor nerves of these smooth muscles are adrenergic. A very low tonic discharge in the adrenergic nerves is sufficient to maintain the muscles in sustained contractile activity

3) In these smooth muscles there also are inhibitory nerves. The neurotransmitter which is released from the inhibitory nerves and which relaxes the muscle cells by acting directly upon them is most probably a substance other than acetylcholine. It is further unlikely that it is histamine, 5-hydroxy tryptamine a prostaglandin or any of the nucleosides, nucleotides or amino acids studied. In the retractor penis a discharge in the inhibitory nerves probably leads rapidly to a high degree of occupancy of receptors for the inhibitory neurotransmitter This may be of importance for erection.

4) The secondary contraction observed after cessation of inhibitory nerve stimulation is probably a true rebound effect and not due to stimulation of a third kind of nerve fibres. The rebound contraction may be of importance for the termination of erection.

5) If cholinergic nerves are present in these smooth muscles, the most probable effect of the presumptive cholinergic fibres is inhibition of adrenergic neurotransmission. It is unlikely that they directly produce the smooth muscle relaxation of erection. Relaxation of the smooth muscles caused by acetyl choline is probably due to nicotinic stimulation of the inhibitory nerves. The mere interruption of tonic adrenergic discharge is not sufficient to produce full penile erection.

6) It is unlikely that posterior pituitary hormones contribute locally to the establishment of penile erection in the bull. This also applies to angiotensin, bradykinin, and Substance P

7) The differences observed in the effects of certain compounds on the retractor penis and the penile artery are due to differences in the muscle cells rather than to differences in their innervation.

ACKNOWLEDGEMENTS

Erik Klinge wishes to express his gratitude to Professor U. S. v. Euler and to Professor B. Andersson, the former and the present Head of the First Department of Physiology, Karolinska Institutet, Stockholm, for generous hospitality during his stay in Stockholm. Nils Sjöstrand wants to thank Professor Matti K. Paasonen, Head of the Department of Pharmacology, University of Helsinki, for kind hospitality during his visits in Helsinki.

The research was supported by grants from the Magnus Bergvall's Memorial Fund (to N. S.), the Yrjö Jahnsson Foundation (to E. K.) and the Swedish Medical Research Council, project number K 72 14X-3767 (to N. S.). Scholarships from the Medical Research Council of the Academy of Finland and the Finnish-Swedish Cultural Fund (to E. K.) have made possible a cooperation for nine months in Stockholm.

For generous supplies of drugs we are indebted to Professor U. S. v. Euler, to Professor S. Bergström and Dr M. Bygdeman, to Dr T. Wadström, to Dr J. H. Sanner, to Dr P. Hedqvist, and to the following companies: ACO AB, AB Astra, Bayer AG, Ciba AG, Draco AB, Ferring AB, Geigy AG, Imperial Chemical Industries Ltd, Medica Ltd, Merck Sharp & Dohme, Pfizer Inc., AB Pharmacia, Sandoz AG, and Star Ltd.

We wish to thank Mrs. Kerstin Westling for careful help in preparing test solutions, Mr E. Linkola for drawing the anatomical pictures, and Mrs. Kiekl Forslind for drawing the graphs. The courtesy of the personnels at the city slaughter houses of Stockholm and Helsinki is gratefully acknowledged.

REFERENCES

Ambache N., The peripheral action of cl. *botulinum* toxin. *J Physiol.* (Lond.) 1949. *108.* 127—141

Ambache N., A further survey of the action of *clostridium botulinum* toxin upon different types of autonomic nerve fibre. *J Physiol.* (Lond.) 1951 *113* 1—17

Ambache N The use and limitations of atropine for pharmacological studies on autonomic effectors. *Pharmacol. Rev* 1955 *7* 467—494.

Anrep, B v and *N Cybulski*, Zur Physiologie der gefässerweiternden und gefässverengernden Nerven. *St. Petersb Med. Wschr* 1884. *20* 215—221.

Armett C J and *J M Ritchie*, The action of acetylcholine and some related substances on conduction in mammalian non-myelinated nerve fibres. *J Physiol.* (Lond.) 1961. *155* 372—384.

Armitage, A. K. and *J H Burn*, The effect of physostigmine on the contraction of the retractor penis muscle of the dog in response to sympathetic stimulation. *Brit. J Pharmacol.* 1967 *29* 218—229

Ashdown, R. R. and *H Pearson*, Anatomical and experimental studies on eversion of the sheath and protrusion of the penis in the bull. *Res. vet. Sci.* 1973. *15.* 13—24.

Ashdown, R. R. S W Ricketts and *R. C Wardley* The fibrous architecture of the integumentary coverings of the bovine penis. *J Anat.* (Lond.) 1968. *103* 567—572.

Ashdown, R. R. and *J A. Smith*, The anatomy of the corpus cavernosum penis of the bull and its relationship to spiral deviation of the penis. *J Anat.* (Lond.) 1969. *104* 153—159

Babulova, A. S. R. Bareggi, A. Bonaccorsi, S Garattini, P L. Morselli and *C Pantarotto*, Correlation between desipramine levels and ( )-noradrenaline uptake and chronotropic effect in isolated atria of rats. *Brit. J Pharmacol.* 1973. *48.* 464—474.

Bacq, Z. M Recherches sur la physiologie et la pharmacologie du système nerveux autonome, XII. Nature cholinergique et adrénergique des diverses innervations vasomotrices du pénis chez le chien. *Arch. int Physiol.* 1935. *40* 311—321.

Bainbridge, J G and *D. M Brown*, Ganglion-blocking properties of atropine-like drugs. *Brit J Pharmacol.* 1960. *15* 147—151.

Baker R. D., N L. Van Demark, C N Graves and *H W Norton*, Effects of pilocarpine and atropine on copulatory behaviour ejaculation and semen composition in the bull. *J Reprod. Fertil.* 1964. *8.* 297—303.

Barger G and *H H Dale*, Chemical structure and sympathomimetic action of amines. *J Physiol.* (Lond.) 1910. *41.* 19—59

Baumgarten, H G B Falck and *W Lange* Adrenergic nerves in the corpora cavernosa penis of some mammals. *Z. Zellforsch.* 1969 *95* 58—67

Beckett, S D., R. S Hudson, D F Walker T M. Reynolds and *R. I Vachon*, Blood pressures and penile muscle activity in the stallion during coitus. *Amer J Physiol.* 1973. *225.* 1072—1075

Beckett, S D R. S Hudson, D F Walker R. I Vachon and *T M Reynolds*, Corpus cavernosum penis pressure and external penile muscle activity during erection in the goat. *Biol. Reprod.* 1972 a. *7* 359—364.

Beckett, S D T M Reynolds R. S Hudson and *R. S Holley* Serial angiography of the crus penis of the goat during erection. *Biol. Reprod.* 1972 b. *7* 365—369.

Bell, C., Autonomic nervous control of reproduction circulatory and other factors. *Pharmacol. Rev* 1972. *24.* 657—736

Bell, C and *J R. McLean*, The distribution of cholinergic and adrenergic nerve fibres in the retractor penis and vas deferens of the dog. *Z. Zellforsch.* 1970 *106.* 516—522.

Bentley G A., Studies on sympathetic mechanisms in isolated intestinal and vas deferens preparations. *Brit. J Pharmacol.* 1962. *19* 85—98.

Birmingham, A. T and A. B. Wilson, Preganglionic and postganglionic stimulation of the guinea-pig isolated vas deferens preparation. *Brit. J Pharmacol.* 1963. *21* 569—580.

Birmingham, A. T and A. B. Wilson, An analysis of the blocking action of dimethylphenylpiperazinium iodide on the inhibition of isolated small intestine produced by stimulation of the sympathetic nerves. *Brit. J Pharmacol* 1965 *24* 375—386.

Bottazzi, F Recherches sur le M. retractor penis et sur d'autres préparations musculaires lisses. *Arch. ital. Biol.* 1916. 65 265—300.

Bowman, W C. and M. T Hall, Inhibition of rabbit intestine mediated by α- and β-adrenoceptors. *Brit. J Pharmacol.* 1970. 38. 399—415

Bozler E, Conduction, automaticity and tonus of visceral muscles. *Experientia* (Basel) 1948. 4. 213—218.

Brücke, E. Th. v Beiträge zur Physiologie der autonom innervierten Musculatur Die elektromotorischen Wirkungen des Musculus retractor penis im Zustande tonischer Kontraktion. *Pflügers Arch. ges. Physiol.* 1910. *133.* 313—340.

Brücke, E. Th. v and S. Oinuma, Beiträge zur Physiologie der autonom innervierten Muskulatur. Über die Wirkungsweise der fördernden und hemmenden Nerven. (Nach Versuchen am M. retractor penis des Hundes.) *Pflügers Arch. ges. Physiol.* 1910. 136. 502—532.

Burgen, A. S. V F Dickens, and L. J Zatman, The action of botulinum toxin on th neuro-muscular junction. *J Physiol.* (Lond.) 1949 *109* 10—24.

Burn, J H., Release of noradrenaline from the sympathetic postganglionic fibre. *Brit. med. J* 1967 2. 197—201

Burn, J H. and M. J Rand, Acetylcholine in adrenergic transmission. *Ann. Rev Pharmacol.* 1965. 5 163—182.

Burnstock, G., Purinergic nerves. *Pharmacol. Rev* 1972. 24. 509—581

Burnstock, G G Campbell, D Satchell and A. Smythe Evidence that adenosine triphosphate or a related nucleotide is the transmitter released by non-adrenergic inhibitory nerves in the gut. *Brit. J Pharmacol.* 1970. *40* 668—688.

Burnstock, G B. Dumsday and A. Smythe, Atropine resistant excitation of the urinary bladder the possibility of transmission via nerves releasing purine nucleotide. *Brit J Pharmacol.* 1972 44. 451—461.

Burnstock, G and C. L. Prosser Conduction in smooth muscles comparative electrical properties. *Amer J Physiol.* 1960 *199* 553—559

Cairnie, A. B., H W Kosterlitz and D W Taylo Effect of morphine on some sympathetically innervated effectors. *Brit. J Pharmacol* 1961 *17* 539—551

Dale, H. H., On some physiological ctions f ergot *J Physiol* (Lond.) 1906 34 163—206.

Dale, H. H., The action of certain esters and thers of choline, and their relation to muscarine. *J Pharmacol. exp. Ther* 1914 6 147—190.

Dale, H H and J H. Gaddum, Reactions of denervated voluntary muscle, nd their bearing on the mode of action of parasympathetic and related nerves *J Physiol* (Lond.) 1930. 70 109—144.

De Zilwa, L. A. E Some contributions to the physiology of unstriated muscle. *J Physiol.* (Lond.) 1901. 27 200—223

Dorr L. D and M J Brody Hemodynami mechanisms of erection in the canine penis. *Amer J Physiol* 1967 213. 1526—1531

Dunk, P J and T Mann, Effect of tropine on the composition f semen and secretory function of male accessory organs in the boar *J Reprod Fertil.* 1963. 5. 101—108.

Dunk, P J and H W Norton, Influence of drugs affecting the autonomi system on seminal jaculation. *J Reprod. Fertil* 1962 4 47—59.

Eakins, K. E and J H. Sanner Prostaglandin ntagonists. In *The Prostaglandins.* Progress in research. Ed. by S. M. M Karim Medical and Technical Publishing Company Ltd, Oxford and Lancaster 1972 pp. 263—292.

Eckhard, C., Untersuchungen uber die Erection des Penis beim Hunde *Beitrage zur Anatomie und Physiologie von C. Eckhard.* III Band. Giessen 1863.

Eckhard, C., Zur Lehre von dem Bau und der Erection des Penis. *Beiträge zu Anatomie und Physiologie von C. Eckhard.* IV Band. Giessen 1869.

Eckhard, C., Ueber den Verlauf der nn. erigentes innerhalb des Rückenmarks und Gehirns. *Beiträge zu Anatomie und Physiologie von C. Eckhard.* VII Band. Giessen 1876.

Edm ds, C. W The point f ttack of certain drugs acting on the periphery II. Action on the retractor peni of the dog. *J Pharmacol. exp. Ther* 1920. 15. 201

Ehinger B. and *B. Falck* Noradrenaline and cholinesterases in concomitant nerve fibres in the rat iris. *Life Sci.* 1963. *4* 2097—2100.
Ehinger B., B. Falck and *B. Sporrong* Possible axo-axonal synapses between peripheral adrenergic and cholinergic nerve terminals. *Z. Zellforsch.* 1970. *107* 508—521
Elliott, *T R.,* The action of adrenalin. *J Physiol.* (Lond.) 1905. *32* 401—467
Eränkö O and *L. Räisänen,* Fibres containing both noradrenaline and acetylcholin esterase in the nerve net of the rat iris. *Acta physiol. scand.* 1965. *63.* 505—506.
Eränkö, O., L. Rechardt, L. Eränko and *A. Cunningham,* Light and electronmicroscopic histochemical observations on cholinesterase containing sympathetic nerve fibres in the pineal body of the rat. *Histochem. J* 1970. *2* 479—489
Euler U S v and *P Hedqvist* Inhibitory action of prostaglandin E_1 and E_2 on the neuromuscular transmission in the *guinea pig vas deferens. Acta physiol. scand.* 1969 *77* 510—512.
Fisher H G Histological structure of the retractor penis muscle of the dog. *Anat. Rec.* 1917 *13* 69—80.
Fletcher W M Preliminary note on the motor and inhibitor nerve-endings in smooth muscle. *J Physiol.* (Lond.) 1898. *2* xxxvii—xl.
Folkow B Impulse frequency in sympathetic vasomotor fibres correlated to the release and elimination of the transmitter *Acta physiol. scand.* 1952. *25.* 49—76.
Folkow B and *E Neil,* In *Circulation.* Oxford University Press, New York, London and Toronto 1971 pp. 14—19.
François Franck, M Recherches sur l'innervation vaso-motorice du pénis. *Arch. Physiol. norm. Path.* (Paris) 1895. *7* 122—153.
Fröhlich, A. and *O Loewi,* Untersuchungen zur Physiologie und Pharmakologie des autonomen Nervensystems. *Naunyn—Schmiedeberg's Arch. exp. Path. Pharmak.* 1908. *59* 34—56.
Furness J B., An examination of nerve-mediated, hyoscine-resistant excitation of the guinea-pig colon. *J Physiol.* (Lond.) 1970 *207* 803—821
Furness J B Secondary excitation of intestinal smooth muscle. *Brit. J Pharmacol.* 1971 *41* 213—226
Fülgraff G v and *L. Schmidt,* Zur Frage der humoral verursachten, atropinresistenten Kontraktion am Pelvicus—Colon—Praparat von Katzen. *Med. exp.* (Basel) 1963. *9* 376—384.
Gaskell, W H On the structure, distribution and function of the nerves which innervate the visceral and vascular systems. *J Physiol.* (Lond.) 1886. *7* 1—80.
Gaskell, W H Do the nervi erigentes leave the spinal cord in anterior or posterior roots? *J Physiol* (Lond.) 1887 *8* iv—v
Gibson, A. and *J S Gillespie* The effect of immunosympathectomy and of 6-hydroxydopamine on the responses of rat anococcygeus to nerve stimulation and to some drugs. *Brit J Pharmacol.* 1973. *47* 261—267
Gillespie J S., The rat anococcygeus muscle and its response to nerve stimulation and to some drugs. *Brit. J Pharmacol.* 1972. *45.* 404—416.
Gillespie J S. and *J C McGrath,* The spinal origin of the motor and inhibitory innervation of the rat anococcygeus muscles. *J Physiol.* (Lond.) 1973. *230* 659—672.
Gillespie, J S and *J C. McGrath,* The response of the cat anococcygeus muscle to nerve or drug stimulation and a comparison with the rat anococcygeus. *Brit. J Pharmacol.* 1974 *50* 109—118
Goldenberg M M Pharmacologic analysis of atropine-fast nicotine responses of the retractor penis and urinary bladder of the cat. *Pharmacologist* 1965 *7* 158.
Goltz, Fr and *A. Freusberg* Ueber die Functionen des Lendenmarks des Hundes. *Pflügers Arch. ges Physiol* 1874. *8* 460—498.
Grabowski, K., Über die Schwellirkume der Harnrohre der männlichen Haussäugetiere unter besonderer Berucksichtigung ihres Bulbusstückes *Veterinary dissertation.* Hannover 1937
Grieten, J and *M A. Gerebtzoff* Les cholinestérases dans l appareil uro-génital. *Ann. Histochim.* 1957 *2* 127—140
Gruber C M The autonomic innervation of the genito-urinary system. *Physiol. Rev* 1933. *13* 497—609.
Gushchin, I. S., Pharmacological analysis of the acetylcholine action on an isolated m. retractor penis in dogs. *Farmakol. i Toksikol.* 1965. *28* 209—
Hart, B L., The action of extrinsic penile muscles during copulatio male dog. *Anat. Rec.* 1972. *173.* 1—6.

Kurt, B. L. and R. L. Kitchell, Penile erection and contraction of penile muscles in the
spinal and intact dog. Amer J Physiol. 1966. 210 257—262.
Holgvist, P Autonomic transmission. In The Prostaglandins. Ed. by P W Ramwell
Plenum Press, New York and London 1973. pp. 101—131
Hedqvist, P and J Brundin, Inhibition by prostaglandin E_1 of noradrenaline release
and of effector response to nerve stimulation in the cat spleen. Life Sci. 1969 8.
389—395.
Heinemann, K., Einige Muskeln des männlichen Geschlechtsapparates der Haussäuge-
tiere (M. bulbocavernosus, M. ischiocavernosus, M. retractor penis) Veterinary
dissertation. Hannover 1937
Henderson, V E. and M. H. Roepke, On the mechanism of erection. Amer J Physiol.
1933. 106. 441—448.
Hillarp, N Å., Structure of the synapse and the peripheral innervation apparatus of
the autonomic nervous system. Acta anat. (Basel) 1946. 2 Suppl. 4
Hillarp, N Å., The construction and functional organization of the autonomic innerva
tion apparatus. Acta physiol. scand. 1959 46. Suppl. 157
Hilton, S. M. and G P Lewis, The relationship between glandular activity bradykinin
formation and functional vasodilatation in the submandibular salivary gland.
J Physiol. (Lond.) 1956. 134. 471—483.
Holman, M. E. and N C. Spitzer Action of botulinum toxin on transmission from
sympathetic nerves to vas deferens. Brit. J Pharmacol. 1973. 47 431—433.
Holmquist, B. and T Olin, Angiography of the internal pudendal artery at electrical
stimulation of the pelvic nerves and at injection of posterior pituitary hormones. An
experimental study of the erectile tissue in the mal dog. Scand. J Urol. Nephrol.
1968. 2 291—296.
Hukovic, S., Responses of the isol ted sympathetic nerve-ductus deferens preparation of
the guinea-pig. Brit. J Pharmacol. 1961 16 188—194.
Hukovic, S. and I. Bubic, Coeur et vaisseaux sanguins isolés avec leurs nerfs comme
moyen de recherche pharmacologique. Path. et Biol. 1967 15. 153—157
Jacobowitz, D and G B. Koelle, Histochemical correlations of acetylcholinesterase and
catecholamines in postganglionic autonomic nerves of the cat, rabbit and guinea pig.
J Pharmacol. exp. Ther 1965. 148 225—237
Johansson, B O Jonsson, J Axelsson and B. Wahlström, Electrical and mechanical
characteristics of vascular smooth muscle response to norepinephrine and isoprotere-
nol. Circulat. Res. 1967 21 619—633.
Kirpekar S. M., J C. Prat, M Puig and A. R. Wakade, Modification of the evoked
release of noradrenaline from the perfused cat spleen by various ions and agents.
J Physiol. (Lond.) 1972. 221 601—615.
Klinge E., Factors influencing the tone f the bull retractor penis muscl M D. Thesis.
University of Helsinki 1969 a.
Klinge E., Noradrenaline and acetylcholinesterase in the nerves of the bull penile artery
Scand. J clin. Lab Invest. 1969 b. 23. Suppl. 108. 35.
Klinge, E Effect of bradykinin on the isolated bull retractor penis muscle. Acta physiol
J clin. Lab Invest. 1969 c. 24. Suppl. 107 77—79
Klinge, E., The catechol amine content of the bull retractor peni muscle Scand
scand. 1970 a. 78. 103—109
Klinge, E., The acetylcholine content and cholinesterase ctivity of the bull retractor
penis muscle. Acta physiol scand. 1970 b. 78 159—167
Klinge E., The effect of some substances on the isolated bull retractor penis muscl
Acta physiol. scand. 1970 c. 78 280—288.
Klinge, E., Is the penis supplied by long or short adrenergic neurons In Abstracts f
volunteer papers. Fifth International Congress on Pharmacology San Francisco,
July 23—28, 1972. p. 127
Klinge, E. and O Penttilä, Distribution of noradrenaline and acetylcholinesterase in
bull and rabbit penile erectile tissue. Ann. Med. exp. Fenn. 1969. 47 17—21
Klinge, E., P Pohto and E. Solatunturi, Adrenergic innervation and structure of the
bull retractor penis muscle. Acta physiol. scand. 1970 78. 110—116.
Klinge, E and P Pohto Innervation of bull retractor penis muscle and peripheral
autonomic mechanism of erection. In Histochemistry of ervous transmission. Ed.
by O. Er[illegible] Progr Brain. Res. 1971 34. 415—421
Kolliker A [illegible] tomische und physiologische Verhalten der
Sexualor[illegible] ch. phys med. Ges. Würzburg 1852. 2. 118

Kuriyama, H., Effects of ions and drugs on the electrical activity of smooth muscle. In *Smooth Muscle*. Ed. by E. Bülbring, A. Brading, A. Jones and T. Tomita. Edward Arnold Publishers Ltd., London 1970. pp. 366—395

Langley J N and *H K. Anderson*, The innervation of the pelvic and adjoining viscera. Parts III—V *J Physiol.* (Lond.) 1895 *19* 85—139

Langley J N and *H K. Anderson*, The innervation of the pelvic and adjoining viscera. Part VI. *J Physiol.* (Lond.) 1896. *20* 372—406.

Lewis, J E D F Walker S D Beckett and *R. I Vachon*, Blood pressure within the corpus cavernosum penis of the bull. *J Reprod. Fertil.* 1968. *17* 155—156.

Lindmar R., K. Löffelholz and *E. Muscholl*, A muscarinic mechanism inhibiting the release of noradrenaline from peripheral adrenergic nerve fibres by nicotinic agents. *Brit. J Pharmacol.* 1968. *23* 280—294.

Löffelholz, K. and E. Muscholl, Inhibition by parasympathomimetic nerve stimulation of the release of the adrenergic transmitter *Naunyn—Schmiedebergs Arch. Pharmak.* 1970 *267* 181—184.

Lovén, Ch., Über die Erweiterung von Arterien infolge einer Nervenerregung. *Berichte über die Verhandlungen der kgl. sächs Gesellschaft der Wissenschaften zu Leipzig, mathem.—physische Classe.* 1866. *18* 85—110.

Luduena, F P Acción de algunos simpaticolíticos (yohimbina, ergotoxina y piperidino metil-3-benzodioxano) sobre el músculo retractor del pene. *Rev Soc. Arg Biol.* 1939 *15* 366—371.

Luduena, F P Modificaciones producidas por la cocaina, en el efecto de algunas aminas sympatomimeticas sobre la presion arterial y el retractor del pene. *Rev Soc. Arg. Biol.* 1940. *16*. 701—735

Luduena F P E. Ananenko O H Siegmund and *L. C Miller* Comparative pharmacology of the optical isomers of arterenol. *J Pharmacol. exp. Ther* 1949 *95* 155—170.

Luduena, F P and *E. O Grigas*, Pharmacological study of autonomic innervation of dog retractor penis. *Amer J Physiol.* 1966. *210* 435—444.

Luduena, F P and *E O Grigas*, Effect of some biological substances on the dog retractor penis in vitro. *Arch. int. Pharmacodyn.* 1972. *196*. 269—274.

MacIntosh, F C R. I Birks and *P B Sastry* Pharmacological inhibition of acetylcholine synthesis. *Nature* (Lond.) 1956. *178*. 1161

Malik, K. U and *M Ling* Modification by acetylcholine of the response of rat mesenteric arteries to sympathetic stimulation. *Circulat. Res.* 1969 *25*. 1—9

Mellander S Comparative studies on the adrenergic neuro-hormonal control of resistance and capacitance blood vessels in the cat. *Acta physiol. scand.* 1960. *50*. Suppl. 176

J Über die organischen Nerven der erectilen männlichen geschlechtsorgane des Menschen und der Säugetiere. *Abhandl. d. kgl. preuss. Akad. d. Wissensch. zu Berlin.* Jahrgang 1835 93—140

Muller L. R., Klinische und experimentelle Studien über die Innervation der Blase, des Mastdarms und des Genitalapparates. *Dtsch. Z. Nervenheilk.* 1902. *21* 86—165

Müller L. R. and *W Dahl*, Die Innervierung der männlichen Geschlechtsorgane *Dtsch. Arch. f klin. Med.* 1912. *107* 113—155

Nedergaard, O A. and *J A. Bevan*, Effects of nicotine dimethylphenylpiperazinium and ganglionic blocking agents at adrenergic nerve endings of the rabbit pulmonary artery *J Pharmacol. exp. Ther* 1969 *168* 127—136.

Nickerson, M Drugs inhibiting adrenergic nerves and structures innervated by them. In *The Pharmacological Basis of Therapeutics*. Ed by L. S. Goodman and A. Gilman. The MacMillan Company New York 1970 pp. 549—584.

Nikolsky W Ein Beitrag zur Physiologie der Nervi Erigentes. *Arch. Anat. Physiol.* (Lpz.) Jahrgang 1879 209—221

Oppenheimer M. J Autonomic control of the retractor penis in the cat. *Amer J Physiol.* 1938. *122* 745—752.

Orlov R. S, On impulse transmission from motor sympathetic nerve to smooth muscle. *Fiziol. Zh. SSSR* 1962. *48* 342—348.

Orlov R. S Spontaneous electrical activity of smooth muscle cells before and after denervation. *Fiziol. Zh.* SSSR 1963 a. *49* 115—121.

Orlov R. S., Transmission of inhibitory impulses from nerve to smooth muscle. *Fiziol. Zh. SSSR* 1963 b. *49* 575—582.

Paton, W D. M The response of the guinea pig ileum to electrical stimulation by coaxial electrodes. *J Physiol.* (Lond.) 1955. *127* 40P—41P

Paton, W D. M., The action of morphine and related substances on contraction and on acetylcholine output of coaxially stimulated guinea pig ileum. *Brit. J Pharmacol.* 1957 12. 119—127

Penttilä, O Acetylcholine, biogenic amines and enzymes involved in their metabolism in penile erectile tissue. *Ann. Med. exp. Fenn.* 1966. 44. Suppl. 9

Penttilä, O and E. Klinge, Action of acetylcholine, biogenic amines and polypeptides on vascular tonus and outflow rate of perfused bovine erectile tissue. *Acta physiol. scand.* 1966. 68. Suppl. 277 159

Penttilä, O and A. Vartiainen, Acetylcholine, histamine, 5-hydroxytryptamine and catecholamine contents of mammalian penile erectile and urethral tissue. *Acta pharmacol.* (Kbh.) 1964. 21. 145—151.

Piotrowski, G Studien uber den peripherischen Gefässmechanismus. *Pflügers Arch. ges. Physiol.* 1894. 55. 240—302.

Prosser C. L., G Burnstock and J Kahn, Conduction in smooth muscle comparative structural properties. *Amer J Physiol.* 1960. 199 545—552.

Reitzel, N L. and J P Long The neuromuscular blocking properties of α, α, dimethyl ethanolamino 4, 4'biacetophenone (hemicholinium) *Arch. int. Pharmacodyn.* 1959 119 20—30.

Root, W S. and P Bard, The mediation of feline erection through sympathetic pathways with some remarks on sexual behavior after deafferentation of the genitalia. *Amer J Physiol.* 1947 151. 80—90.

Schaeppi, U R. L. Donaldson and M Dodd, Nonganglionic parasympathomimetic ction of nicotine upon the isolated cat iris. *J Pharmacol. exp. Ther* 1966. 154. 216—223.

Schaumann, W Inhibition by morphine of the release of cetylcholine from the intestine of the guinea-pig. *Brit. J Pharmacol.* 1957 12 115—118.

Seidel, G E. and R. H. Foote, Motion picture analysis of ejaculation in the bull. *J Reprod. Fertil.* 1969 20 313—317

Semans, J H and O R Langworthy Observations on the neurophysiology of sexual function in the male cat. *J Urol.* 1938. 40. 836—846.

Sertoli, E., Contribution à la physiologi générale des muscles lisses. *Arch. ital. Biol.* 1883. 3. 78—94.

Signoret, J P Action de l atropin sur le comportement sexuel et la composition du sperme chez le taureau. *Ann. Biol. anim. Bioch. Biophys.* 1962. 2. 163—166.

Sjöstrand, N O Effect of some smooth muscle stimulants on the motor response of the isolated guinea pig vas deferens to hypogastri nerve stimulation. *Nature* (Lond.) 1961. 192 1190—1191

Sjöstrand, N O Inhibition by ganglionic blocking agents of the motor response of the isolated guinea-pig vas deferens to hypogastric nerve stimulation. *Acta physiol. scand.* 1962. 54. 306—315.

Sjöstrand, N O The adrenergic innervation of th vas deferens and the accessory male genital glands. *Acta physiol. scand.* 1965. 65 Suppl. 257

Sjöstrand, N O A note of th dual effect of prostaglandin E_1 on the responses of the guinea pig vas deferens to nerve stimulation. *Experientia* (Basel) 1972. 28. 431—432.

Sjöstrand, N O., Effects of acetylcholine and some other smooth muscle stimulants on the electrical and mechanical responses f the guinea-pig vas deferens to nerve stimulation. *Acta physiol. scand.* 1973 a. 89. 1—9

Sjöstrand, N O Effects of adrenaline, noradrenaline and isoprenaline on the electrical and mechanical responses of the guinea-pig vas deferens to nerve stimulation. *Acta physiol. scand.* 1973 b. 89 10—18.

Sjöstrand, N O and G Swedin, Potentiation by smooth muscle stimulants of the hypogastri nerve-vas deferens preparation from normal and castrated guinea-pig. *Acta physiol. scand.* 1968. 74. 472—479

Spina, A., I.—II. Experimentelle Beitrage zu der Lehre von der Erection und Ejaculation. *Wien med. Blatter* 1897 No 10—13. 180—211.

Szerb J C The effect of morphine on the adrenergic nerves of the isolated guinea-pig jejunum. *Brit. J Pharmacol.* 1961. 16. 23—31.

Thielen, P M. Renders and D. Rectem, Régions médullaires contrôlant l'érection et la rétraction du pénis chez le lapin. *Arch. int. Physiol.* 1969. 77 340—342.

Thoenen, H W Haefely and H. Staehelin, Potentiation by tetraethylammonium f the response of the cat spleen to postganglionic sympathetic nerve stimulation. *J Pharmacol. exp. Ther* 1967 157 532—540.

Tranzer J P and H Thoenen, Significance of 'empty vesicles' in post ganglionic sympathetic nerve minals. *Experientia* (Basel). 1967 23 123—124.

Ursillo R. C. and *B B. Clark*, The action of atropine on the urinary bladder of the dog and on isolated nerve-bladder strip preparation of the rabbit. *J Pharmacol. exp. Ther* 1956. *118*. 338—347

Vanhoutte, P M. and *J T Shepherd*, Venous relaxation caused by acetylcholine acting on the sympathetic nerves. *Circulat. Res.* 1973. *32* 259—267

Volle, R. L. and *G B Koelle* Ganglionic stimulating and blocking agents. In *The Pharmacological Basis of Therapeutics*. Ed. by L. S. Goodman and A. Gilman. The MacMillan Company New York 1970. pp. 585—600.

Watson, J W., Mechanism of erection and ejaculation in the bull and ram. *Nature* (Lond.) 1964. *204* 95—96.

Westfall, D P., Antagonism by protriptyline and desipramine of the response of the vas deferens of the rat to norepinephrine, acetylcholine and potassium. *J Pharmacol. exp. Ther* 1973. *185* 540—550.